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Spectral characteristics and biological activity of synthetic humic-like substances on the basis of carbohydrates

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Abstract. The study of the structure and properties of synthetic analogues of humic substances is an important research task. This paper presents the results of studying the structural and group composition of the products of water-soluble fractions synthesized in the *D*-glucose - *m*-aminobenzoic acid and *D*-glucose - *p*-toluidine reaction systems using electronic and vibrational spectroscopy. The IR-Fourier transform spectroscopic study shows the similarity of the structural-group composition of the selected products with those for humic and humic-like substances of different origin. The study of growth-regulating ability confirms its presence for the products of both systems, while the aromatic fragments of *m*-aminobenzoic acid impart higher stimulating activity to the substances. Analysis of the structural-group composition of the products obtained in the framework of computer modelling suggests their potentially non-toxic nature, which makes further research in this direction promising.

1. Introduction

Interest in synthetic analogues of humic substances is explained by the possibility of obtaining drugs with desired properties [1], [2]. Due to the established and proven practically significant properties of humic substances, various methods for their preparation and the interrelation of structure and properties are currently being intensively investigated, and chemical and biochemical methods for the preparation of synthetic analogs of humins have been developed to date.

The variety of known properties of humic substances is quite high: antioxidant, antiviral, surface-active, growth-regulating. Information about the recruitment of the main atomic groups in the composition of the products, obtained by the method of IR-Fourier spectroscopy in the study of the structural-group composition of humic substances, is a reliable basis for classifying the studied products to a specific type of drugs [3].

Analysis of the literature data shows the presence of correlation dependences established on the basis of the theory of computer recognition of images, linking the structure, activity and toxicity of plant growth regulators [4]. In the latter case, a certain role is noted for the derivatives of *m*- and *p*-disubstituted arylamines [5]. But the literature data are often contradictory. On the other hand, researchers pay special attention to systems based on simple carbohydrates [2], which are reactive and available substrates. In this regard, the purpose of this work was to carry out a spectral study of the structure of the products of water-soluble fractions isolated from *D*-glucose - *m*-aminobenzoic acid (*m*-ABA) and *D*-glucose - *p*-toluidine reaction systems, as well as to study the possibility of using them as available and promising plant growth regulators.

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2. Materials and methods

The target products were synthesized by thermostating equimolar (0.002 mol) mixtures of *D*-glucose - arylamine ("analytical grade") in 20 ml of solvent (dried ethanol, "Merck") for 2 hours at 70 ° C in reflux flasks. At the end of the reactions, after the solvent was removed, the solids were fractionated with water (some of the solutions were moved by dialysis), the products of water-soluble fractions were also isolated in solid form in the form of brown powders and dried, after which they were used in bio-experiments.

The structural group composition of the synthesized products was studied by the method of IR-Fourier transform spectroscopy. The spectra of solid products were taken in the transmission format in KBr tablets (1:250) on FSM 2201 FT-IR spectrometer (InfraSpek LLC, St. Petersburg, Russia) in wave interval figures of 3500-500 cm^{-1} with a spectral resolution of 4 cm^{-1} at 40 scans, the baseline was approximated by a 4-order polynomial. The original and differential spectra were processed in FSpec program. Electronic spectra of aqueous solutions were recorded with an SF-2000 spectrophotometer (OKB Spectr, St. Petersburg, Russia) in the wavelength range of 300-600 nm in quartz cuvettes. For semi-quantitative characteristics, differential spectra and absorption indexes were used. Bioexperiments were carried out on seeds of a Classic cucumber variety according to the method [5], after four days of incubation at 250 °C; the number of germinated seeds and the degree of germination were evaluated in comparison with the control sample.

3. Results and discussions

The FTIR spectra of the solid products of the water-soluble fractions of both systems under study (figure 1A) show the presence of an aliphatic component, in particular, the C-H symmetric and asymmetric stretching vibrations bands (2850, 2922 cm^{-1}), while demonstrating a number of significant differences.

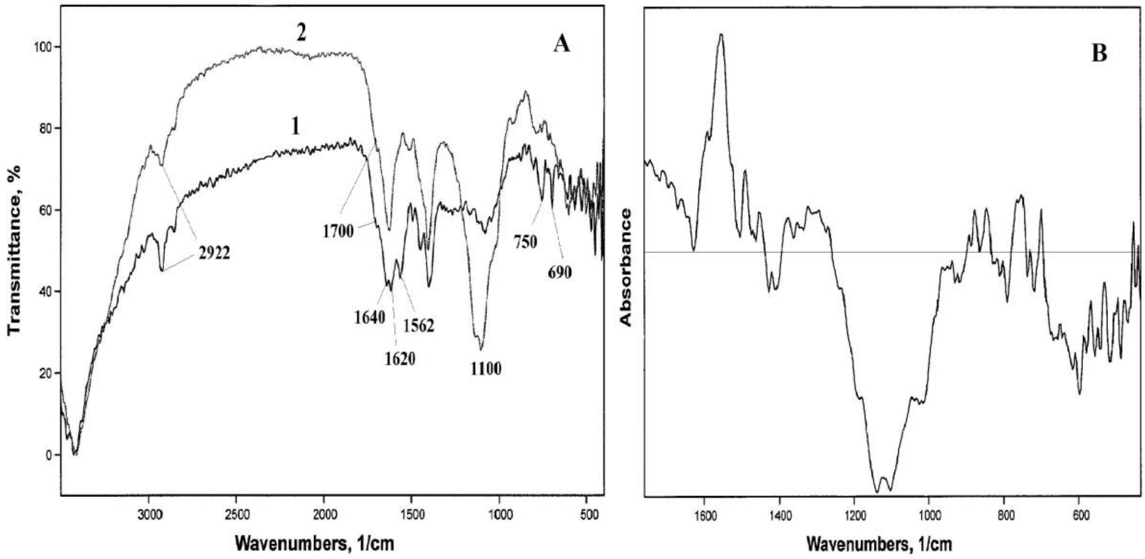

Figure 1. FTIR spectra of products of water-soluble fractions: 1 - *D*-glucose - *m*-ABA; 2 - *D*-glucose - *p*-toluidine (A); Difference profile of FT-IR spectra (1/2) of systems (B).

Quite intense peaks at 1400, 1540–1560 cm^{-1} ($\nu^{s,as}_{COO^-}$) are also recorded; carbonyl bands at 1700 cm^{-1} are found in the form of weak arms, which is characteristic of C=O-groups, which appear on the background of the condensed structure [2, 6, 7], as well as the zwitterionic structure of the amino carboxylate fragments.

Low-frequency bands at 690, 750 cm^{-1} ($\gamma_{=C-H}$) are recorded in the spectrum of the product of the system with *m*-aminobenzoic acid, while there is a shift in them characteristic of ABA fragments in the structure of high-molecular products [6]. Mid-frequency bands of 1490, 1562, 1640 cm^{-1} ($\nu_{C=C}+\delta_{C-H}$) also indicate the presence of an aromatic component in the structure of the product in *D*-glucose - *m*-ABA system [6]. Weak peaks at 1080, 1160, 1207, 1360 cm^{-1} relate to vibrations of O-C, N-C-condensed structures, whereas the signal at 1620 cm^{-1} can be attributed to stretching vibrations of C = N fragments [5]. Weak absorption in the region of 1580-1490 cm^{1} is typical of secondary aromatic amines [7], a weak shoulder at 1510 cm^{-1} corresponds to δ^{s}_{NH} [1] and is characteristic of secondary arylamino groups [8]. Signals in the region of 1560-1640 cm^{-1} can be attributed to asymmetric deformation N-H vibrations [1, 9], and the vibration bands of aromatic rings can be superimposed on them [10]. These data indicate different ways of binding fragments of *m*-aminobenzoic acid into the product structure (secondary amino group, azomethine).

In the spectra of products isolated from *D*-glucose – toluidine system, bands are recorded that show predominantly aliphatic functionalization, and the signal in the range of 800–860 cm^{-1}, attributable to out-of-plane deformation vibrations of *p*-disubstituted arenes, is not fixed. Differences in the structural group composition are clearly visible when analyzing the profile of the bands of the difference spectrum in the absorption format (figure 1B) [9]. The maxima in the regions of 1550-1620 and 700-900 cm^{-1} confirm the aromatic component of the products of *D*-glucose system - *m*-ABA, while the minima in the region of 1000-1200 cm^{-1}, corresponding to complex $\nu_{COC}+\delta_{COC}+\nu_{CCO}$-vibrations, indicate aliphatic O-C-functionalization of products of the second system [9].

The difference in the functional composition of the products may be due to different mechanisms of the initial stages of amino-carbonyl interactions in the systems under study. Due to the low donor properties of the *m*-ABA amino group, the Amadori rearrangement is difficult, and therefore a more favorable reaction direction suggests a direct retro-aldol disintegration of primary N-aminoconjugates along C_2-C_3(C_3-C_4) bonds, realized in ethanol media [11]. Such decomposition products are known to be active with respect to subsequent condensation processes, including with arylamines, which determines the further development of the formation of the product structure and the binding of *m*-ABA to both water-insoluble and water-soluble fractions. In this case, based on the data of [12], the described mechanism of the formation of the structure (figure 2) can be represented as follows (Ar ≡ *m*-carboxyphenyl):

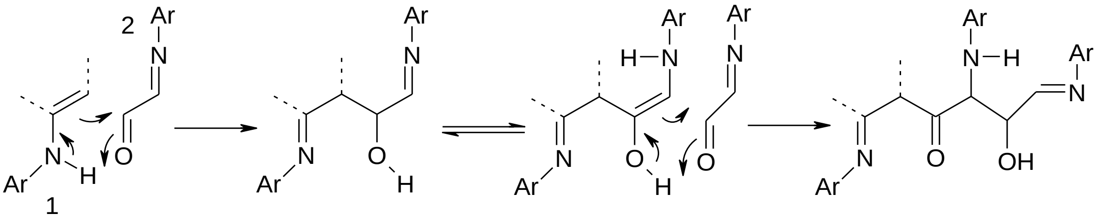

Figure 2. The proposed mechanism for the formation of the structure of products in the system with *m*-ABA.

N-aminoconjugate 1 in the enamine form reacts with the C_2-imino-derivative 2, which is formed in advance from 1 as a result of retro-aldol disintegration [11]. According to a similar scheme, subsequent reactions are likely to occur, leading to products of irregular structure, capable of further complication (condensation, dehydration). Nevertheless, the ways of direct interaction of free arylmine with O=C–C=C centres are not excluded [8].

In a system with more basic *p*-toluidine, the Amadori rearrangement with subsequent elimination of arylamine and the disproportionation reaction with preservation of the carbohydrate chain, whose products complicate the structure through condensation processes, are the most likely processes [13], while *p*-toluidine is bound in substances of water-insoluble fractions. The formation of water-soluble products in this case proceeds, probably, in a manner close to that described above involving carbonyl (enol) functions, with the formation of nitrogen-free derivatives. The total intensity of the sugar reactions in this system is higher than in the system with *m*-ABA, since the rate of formation of

Amadori products significantly exceeds the rate of formation of C_2-intermediates [11]. Summarizing the data on the structural-group composition of the selected products, we can conclude that there are differences in the structure of the products: the water-soluble fractions of *D*-glucose – *m*-aminobenzoic acid system contain condensed products with related *m*-ABA fragments, while the products of *D*-glucose system are *p*-toluidine represented by substances of mainly non-aromatic character.

Electronic spectra (figure 3, spectra 1, 2) show continuous absorption in almost the entire visible region, which indicates the presence of conjugated bond systems. Analysis of the spectra profiles also shows the difference in the optical properties of the systems.

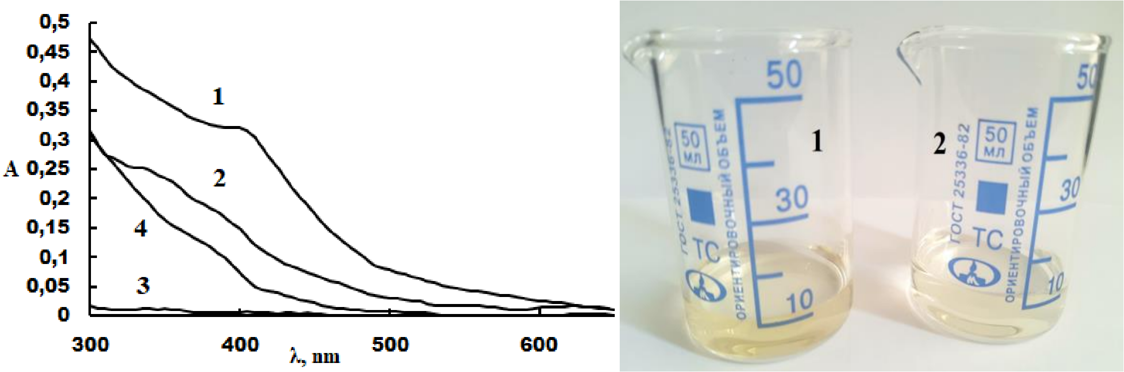

Figure 3. Electronic spectra and appearance of water-soluble fractions: 1 - *D*-glucose - *m*-ABA (E_{350}/E_{450} = 2.1); 2 - *D*-glucose - *p*-toluidine (E_{350}/E_{450} = 3.4); 3 - *D*-glucose - *m*-ABA (dialysate); 4 - *D*-glucose - *p*-toluidine (dialysate).

In the spectrum of *D*-glucose – *m*-aminobenzoic acid system, a relatively sharp maximum at 400 nm is recorded, whereas for *D*-glucose – *p*-toluidine system, two inflections are recorded at 330 and 380 nm. The values of the E_{350}/E_{450} indices, which allow an approximate estimate of the aliphatic component of humic substances, were calculated from the absorption at 350 and 450 nm (figure 3, spectra 1, 2). According to the authors of [14], with an increase in the values of E_{350}/E_{450}, the aliphatic component of humic substances increases, which is consistent with the findings based on the results of IR Fourier transform spectroscopy. The spectra of the solutions after 24 hours of dialysis into distilled water (figure 3, spectra 3, 4) show a different ratio of low molecular weight stained products in the fractions: in *D*-glucose - *m*-ABA system, the colored products are more condensed.

Evaluation of growth-regulating properties was carried out using solutions of products of water-soluble fractions (figure 4), while the data in table 1 show a higher stimulating activity of products of *D*-glucose system - *m*-ABA. The high growth-stimulating ability of systems with high aromatic content was also noted by other authors [15]; the effect of the products of *D*-glucose –*p*-toluidine system on growth is also probably due to their functionalization in a complex way. A detailed study of the mechanisms of interaction of components of humic substances with the active centers of plant substances is rather difficult and continues to be the subject of discussion [15].

Table 1. The influence of the synthesized products on the morphogenesis of seeds of cucumber of Classic variety.

System	The concentration of solutions, %	Germination, %	The average length of seedlings, cm
Control (water)	-	100	2.28 ± 0.05
D-glucose – *p*-toluidine	0.001	100	2.44 ± 0.01
D-glucose – *м*-ABA	0.001	100	3.10 ± 0.05

Figure 4. Cucumber seedlings of Classic variety after 4 days of temperature control in the dark: A - control sample; B - processing by the products of *D*-glucose - *p*-toluidine system; C – processing by the products of *D*-glucose - *m*-ABA system.

It is known that a number of aromatic amines are quite toxic substances [8], while it is rather difficult to establish their influence in the composition of complex products on specific biological objects reliably. Nevertheless, from the general point of view, based on the well-known correlations “structure-activity-toxicity” [4], it is possible to estimate roughly the relationship of the structural-group composition of the synthesized products with their activity. Evaluation is based on informative values of -1 <r <+1, while the condition r> 0.1 corresponds to an increase in activity, while r <-0.1 is a decrease in the activity of the manifestation of the analyzed property. Thus, the secondary amino group forms optimal for its effect on growth-regulating activity (*r*) and toxicity (*r’*) of fragments with *m*-substituted phenyl and ethylene group [4] (figure 5):

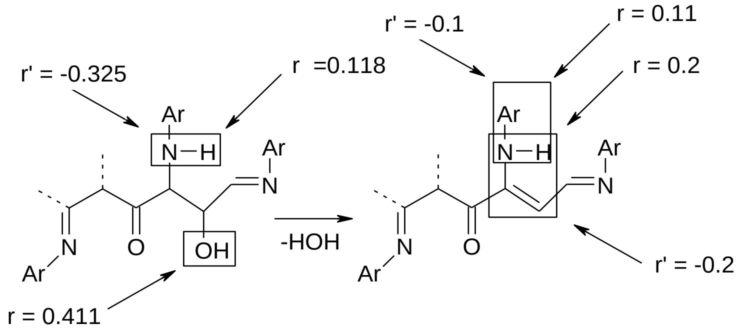

Figure 5. Evaluation of the structural features of the products of *D*-glucose - *m*-ABA system in relation to growth-regulating activity and toxicity.

Other functions (tertiary nitrogen, imino, oxo, hydroxy groups) also make a definite contribution based on known models [4], while selecting the experimental conditions one can control the ratio of functions in the final structure to a certain extent. In this connection, it is reasonable to assume that the products obtained by us are not including a combination of functional groups that enhance toxicity, which makes further research in this direction promising.

4. Conclusion

Thus, based on the analysis of the spectral characteristics of the products of water-soluble fractions synthesized in *D*-glucose - *m*-aminobenzoic acid and *D*-glucose - *p*-toluidine systems, their structural-group composition, similar to that for humic substances of different origin, was established. Due to the different functionality, the products obtained show different activity as growth regulating agents: the products of the first system show a higher stimulating effect, probably due to the greater aromaticity. The technology for obtaining and isolating the products studied is quite simple, which implies their availability. Model representations based on pattern recognition theory show combinations of functional groups in products that are favorable for growth-regulating activity and low toxicity.

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