took place slowly and was not complete after two days. On heating (80°C) the diisoeugenol spot appeared on the plate immediately, and the reaction was complete in 40 min.

Consequently, in the presence or in the absence of guaiacol, on heating or at room temperature, guaiacylpropan-1-ol undergoes dimerization in an acid medium with the splitting out of water and the formation of diisoeugenol.

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PRODUCTION OF WATER-SOLUBLE PHYTOMELANINS

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Phytomelanins (PMs) possess a unique combination of physicochemical properties (antioxidant, semiconducting, electron- and ion-exchange, chelating, catalytic) and a many-sided biological activity (antitumoral, radioprotective, lysis-preventing, growth-activating, bacteriostatic) [1, 3]. They belong to a promising class of physiologically active compounds that permit the creation of combined drugs with a prolonged effect [2]. However, the possibilities of using PMs are limited by the absence of cheap sources of their production. Another serious factor complicating the structural-functional study of PMs and their practical application is the insolubility of PMs in organic solvents and in water. Furthermore, the necessity has arisen in acute form for the development of a series of unified methods of obtaining various types of melanins in which all the conditions of their isolation are standardized. Such an approach is necessary since, in view of the polymeric nature of the pigments, it will permit melanins with reproducible properties to be obtained.

In continuation of investigations pursued earlier [3], we have developed a general principle for the isolation of PMs from diverse natural raw materials which ensures the production of the water-soluble NH_4 salt of the substance (solubility about 20%) with a yield of 0.6-0.9%. As raw materials we have used valves from the cotton plant *Gossypium hirsutum*, bagasse from sugar-beet *Beta vulgaris*, pomace from the grape *Vitis vinifera*, and seed husks from the sunflower, *Helianthus annuus*, i.e., wastes from the corresponding industries that may be considered as promising raw materials for obtaining PMs.

The production of the PMs comprised: 1) extraction with 5% NH_4OH at a liquor ratio of 1:8-1:10, 25°C; for raw material containing more than 3-5% of pectin we used 5% NaOH as extractant, at a liquor ratio of 1:10-1:15; 2) precipitation with dilute HCl (1:1) at pH 3.0-3.5; 3) washing with H_2O to pH 6.0-6.5; 4) extraction with 1% NH_4OH; 5) reprecipitation with HCl (1:1); 6) reextraction with 1% NH_4OH; 7) fractional precipitation with C_2H_5OH from ammoniacal solutions at pH 8.5-9 (a fraction was collected at a ratio of the volume of the precipitant to the volume of the solvent of 9:1); 8) dissolution in H_2O; 9) gel filtration on Sephadex G-25 (eluent 0.001% NH_4OH, pH 9.8). The eluate was concentrated in a rotary evaporator.

In the $NAD \cdot H_2$ ($0.72 \cdot 10^{-4}$ M)–($K_3Fe(CN)_6$ ($3.65 \cdot 10^{-4}$ M) system the purified preparations of PMs (0.25 mg) in phosphate buffer (pH 7.2) accelerated electron transfer by a factor of 2.5-3.0. The process of reducing Fe^{3+} is described by the following equation, with the

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equilibrium constant $\log K = 4.0\text{-}4.1$ at 25°C:

$$2Fe^{+2} + PM\cdot(OH)_2 \overset{K}{\rightleftharpoons} 2Fe^{+2} + PM\ (O_2) + 2H^+,$$

where $PM(OH)_2$ and $PM(O)_2$ are, respectively, the reduced (phenolic) and oxidized (quinoid) forms of the PMs.

Spectra of the PMs in the UV and visible region (230-800 nm): in 0.05 M NaOH, a monotonic decrease in the optical density A with an increase in the wavelength λ; in the coordinates $\log(A\cdot10^3)-\lambda$, the spectrum formed a straight line with an angle inclination to the wavelength axis, α, given by $\tan\alpha = -(0.0026\text{-}0.0032)$.

A difference UV spectrum (pH 4 against pH 10) revealed the absorption of phenolic (λ 295 nm) and quinoid (λ 246 nm) groups.

IR spectra (ν_{max}^{KBr}, cm^{-1}): 3400 (H-bound OH; 1.38 ± 0.08); 1600 (C=C; COO^-–asymm., 1.00); 1400 (COO^-–symm., 0.54 ± 0.04); 1100 (C–O–C–asymm., C–OH; 0.83 ± 0.04). The average positions and relative intensities of the most characteristic bands are given. After HCl treatment, a band appeared at 1710 cm^{-1} (COOH) with a simultaneous decrease in the intensity of the bands at 1600 and 1400 cm^{-1}.

ESR spectra: singlet slightly asymmetric signal with a value of the g-factor of spectroscopic splitting close to the g-factor of a free electron (2.0023), broad line of resonance absorption with $\Delta H = 4.0\text{-}5.2$ Oe and an intensity corresponding to a concentration of paramagnetic centers of $5\cdot10^{17}\text{-}4\cdot10^{18}$ spins/g of dry substance. The shape of the line was intermediate between Gaussian and Lorentzian. The times of spin-lattice (T_1) and spin-spin (T_2) relaxation at 25°C were, respectively, $(1.1\text{-}4.2)\cdot10^{-4}$ and $(1.5\text{-}3.5)\cdot10^{-6}$ s, i.e., $T_1 \gg T_2$.

Thus, the isolation of PMs from plant raw material under the above standardized conditions with fractional precipitation of the PMs by ethyl alcohol from ammoniacal solutions enables water-soluble (about 20%) preparations to be obtained with a high reproducibility of their properties.

The results obtained, creating the prerequisites for a further study of structure–properties interrelationships in the phytomelanin series, will enable the chemical and biological activities of the pigments to be varied and will open up new possibilities for their wide practical employment.

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