ANTHOCYANINS OF HYBRID HIBISCUSES

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UDC 547.972/73

The flowers of many hybrid forms of hibiscus obtained by the interspecies hybridization of the North American species of hibiscus Hibiscus moscheutos, H. militaris, and H. coccineus contain considerable amounts of anthocyanins [2].

From the flowers of H. mutabilis L. we have previously isolated cyanidin 3,5-diglucoside [3], and from the flowers of H. esculentus L. we have isolated cyanidin 4'-glucoside and cyanidin 3-glucosido-4'-glucoside, which are very rarely found in plants [4].

Continuing our study of the anthocyanins of plants of the genus Hibiscus, we have found two anthocyanins in the flowers of hybrid hibiscuses (M. Gor'kii, Gladiolus vidnyi, Yu. Gagarin, Alemsheka, Kolkhoznitsa, Krasnyi partizan, and Kyzyl Uzbekistan). However, the amounts of some anthocyanins in different species of hybrid hibiscus are different.

The individual anthocyanins of the hybrid hibiscus M. Gor'kii were separated and isolated on a column of cellulose powder by the method described previously [5]. This gave two crystalline anthocyanin glycosides with mp 239-241°C and 215-217°C (decomp.).

The anthocyanin with mp 239-241°C had the composition C_{26}H_{29}O_{11}Cl·4H_{2}O; R_f 0.36 (systems 1 and 2), λ_{max} 523 nm (E_{1%} 2.2636) (0.01% HCl, methanol), and on the addition of a 5% solution of aluminum chloride in ethanol, it had λ_{max} 558 nm, which is characteristic for anthocyanins containing free hydroxy groups in the C-3' and C-4' positions [6]. This anthocyanin appeared on a chromatogram in ordinary light in the form of a dark pink spot and in UV light it gave a red fluorescence, while in the presence of ammonia vapor and when the chromatogram was sprayed with a 1% aqueous solution of sodium carbonate it became bright blue.

To determine the nature of this anthocyanin we performed acid, enzymatic, and stepwise acid hydrolyses and also oxidative degradation with hydrogen peroxide. The acid and enzymatic hydrolyses of the anthocyanin formed the aglycone - an anthocyanidin - and sugars - glucose and xylose.

The melting point of the anthocyanidin was above 300°C (decomp.) and it had the composition C_{15}H_{11}O_{4}Cl, λ_{max} 535 nm, shifting to 572 nm on the addition of aluminum chloride, which shows the presence of hydroxy groups in its side chain. When the anthocyanidin was chromatographed on paper in systems 3 and 4, its R_f values (0.69 and 0.54) were the same as for the cyanidin isolated from the flowers of the cotton plant.

The alkaline cleavage of the aglycone with 15% barium hydroxide at 100°C for 30 min led to the formation of phloroglucinol and protocatechuic acid, which were identified by paper chromatography in the presence of markers.

The stepwise acid hydrolysis of the substance with 3.5% hydrochloric acid in methanol for 15 minutes gave chrysanthemin and xylose, and further hydrolysis gave cyanidin and glucose.

To determine the ratio of aglycone and sugar in the glycoside, it was subjected to complete acid hydrolysis. The amount of cyanidin (aglycone) formed was determined spectrophotometrically at a wavelength of 535 nm. The calculation of the proportion of aglycone was made by means of a calibration curve plotted for pure cyanidin. It was found that the percentage of cyanidin in the molecule of the glycoside was 52, which corresponds to an aglycone: sugar ratio of 1:2.

The ratio of the sugars to one another was found by the aniline phthalate method [7], for which purpose the hydrolyzate obtained after the complete acid hydrolysis of the glycoside was chromatographed in system 1. The sugars were eluted from the chromatogram with glacial acetic acid. The intensity of the coloration of the solution was measured on an FEK-M photoelectric colorimeter. The amounts of sugars were determined by means of calibration curves plotted for pure samples of glucose and xylose. It was found that the ratio of glucose to xylose was 1:1.

The results of the determination of the site of attachment of the sugar in the anthocyanin by oxidative degradation [8] showed that the sugar of this anthocyanin is a bioside and is attached to the cyanidin at C₃. On further acid hydrolysis, the bioside gave glucose and xylose.

To determine the position of the bond between the sugars in the bioside we used the color reactions of disaccharides [9]. The bioside isolated from the anthocyanin under investigation formed with diphenylamine-urea a pink spot which became orange-brown after 30 min. The diphenylamine-p-anisidine spot of the bioside was blue-green, becoming deep blue after 24 h. This shows that the glucose and the xylose are connected in the bioside by a 4'1 bond.

The IR spectrum of the anthocyanin showed absorption bands in the following regions (cm⁻¹): 3300-3400 (OH), 1650 (pyran ring), 1610-1430 (aromatic ring), 1200, 1080, and 1040 (C-O), 890 (β-glycosidic bond).

The differential IR spectrum of the glycoside showed three maxima in the 1010, 1040, and 1080 cm⁻¹ regions, which gives grounds for assuming that the sugars are present in the pyranose form.

The enzymatic glycolysis of the anthocyanin with mp 239-241°C by means of the enzyme of Aspergillus oryzae gave a hydrolyzate containing glucose and xylose, which shows β linkages between the aglycone and the glucose and between the glucose and the xylose. In addition, the presence of a β linkage both between the aglycone and the sugar and also between the monosaccharides was confirmed by the presence of an absorption band in the 890 cm⁻¹ region of the IR spectrum of this anthocyanin.

What has been said above enabled the anthocyanin with mp 239-241°C to be characterized as cyanidin 3-β-D-xylopyranosyl-(1→4)-β-D-glucopyranoside. This glucoside is a new one which has not been described in the literature, and we have called it gossypicyanin.

The second anthocyanin, with mp 215-217°C (decomp.) has the composition C₂₁H₂₁O₁₁Cl.₂H₂O, Rf 0.23 (system 1) and 0.36 (system 2). λmax 525 nm (Ε 1% cm⁻¹ 2,2613) shifting on the addition of a solution of aluminum chloride to λmax 575 nm. On a chromatogram the anthocyanin gave a pink coloration and in UV light it appeared violet. The spot of the anthocyanin was colored blue by a solution of sodium acetate and by ammonia vapor.

On the basis of acid and enzymatic hydrolyses, oxidative degradation, and the alkaline fusion of its aglycone, and also its UV and IR spectra, the second anthocyanin was identified as chrysanthemin – cyanidin 3-β-D-glucopyranoside [10]. Chrysanthemin and cyanidin 3-β-D-xylopyranosyl-β-D-glucopyranosides have also been isolated from other species of hybrid hibiscuses (Gladiolus vidnyi, Alemushka, Kolkhoznitsa, Krasnyi partizan, Yu, Gagarin, and Kyzyl Uzbekistan).

**EXPERIMENTAL**

The following solvent systems were used in the study of the composition of the anthocyanins and in their separation: 1) water–acetic acid–hydrochloric acid (82:15:3); 2) butan-1-ol–acetic acid–water (4:1:5); 3) butan-1-ol–2 N hydrochloric acid (1:1, upper phase); 4) acetic acid–hydrochloric acid–water (5:1:5); 5) ethyl acetate–pyridine–water (2:1:2); and 6) butan-1-ol–benzene–acetic acid–water (2:10:2:1).

Preparation of the Combined Anthocyanins. The air-dry comminuted flowers of hybrid forms of hibiscuses of the variety M. Gor'kii collected at the beginning of August (1 kg) were extracted with chloro-