ANTHOCYANINS OF Hibiscus cannabinus

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A widely cultivated plant of the genus Hibiscus (family Malvaceae) is Hibiscus cannabinus var. simplex (Kenaf hemp). In spite of the fact that the flavonol glycosides of Kenaf hemp have been studied fairly well [1, 2], the anthocyanins of this plant have not been investigated.

The results of the spectrophotometric determination of the content of anthocyanins in various organs of four species of the genus Hibiscus grown in the experimental section of the Central-Asian branch of VIR [All-Union Scientific-Research Institute of Plant Breeding] showed that the largest amount of anthocyanins is present in the flowers of Hibiscus cannabinus var. simplex [3]. The paper chromatography of extracts of the plants studied showed the presence of two anthocyanin spots.

To isolate the anthocyanins, flowers of Kenaf hemp were first treated with chloroform. Then the anthocyanins were extracted at room temperature with methanol containing 1% of hydrochloric acid. The anthocyanins were precipitated from the concentrated methanolic extract with diethyl ether.

The individual substances were separated by partition chromatography in a column filled with cellulose powder using solvent system 1 [water–acetic acid (85 : 15)]. After chromatography, the sharply separated zones were cut out, and the anthocyanins were eluted separately with 0.01% hydrochloric acid in methanol.

The concentrated eluate of the lower zone of the column yielded an anthocyanin with mp 164-166°C (decomp.). The UV spectrum of the substance had λ_{max} 531 nm. The addition of 5% aluminum trichloride (in ethanol) led to a bathochromic shift of the maximum to 576 nm, showing the presence in the anthocyanin of free hydroxy groups at C-3' and C-4'.

The stepwise acid hydrolysis of the glycoside formed delphinidin 3-glucoside and xylose, while complete acid hydrolysis yielded delphinidin, glucose, and xylose.

The oxidation of the anthocyanin with hydrogen peroxide [4] showed that the sugar was attached to the aglycone in the C-3 position in the form of a bioside. The identity of the aglycone with delphinidin was shown by their direct comparison. For this purpose we used delphinidin isolated from the skin of Solanum melongena L. (garden eggplant) [5]. Cleavage of the glycosides by the enzyme of Aspergillus oryzae showed a β linkage between the aglycone and the sugar. Thus, the first anthocyanin is a new substance which can be be characterized as delphinidin 3-β-D-xylosyl-β-D-glucoside, and we have called it cannabinin (I).

In the same way, the eluate from the upper zone of the column yielded a second anthocyanin which, on full acid hydrolysis, formed delphinidin and glucose.

The results of a study of the products of acid and enzymatic hydrolysis, oxidative degradation with hydrogen peroxide, and alkaline cleavage of the aglycone permitted the second anthocyanin to be identified as 3-β-D-glucoside, which is known in the literature under the name of myrtillin (II).

The ratio of cannabin to myrtillin in the total anthocyanin of Kenaf hemp is approximately 4 : 1.

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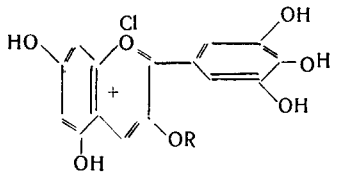

I. R–xylosylglycoside
II. R–glucoside.

EXPERIMENTAL

The elementary analyses of all the compounds corresponded to the calculated figures for C, H, and Cl.

Isolation of the Anthocyanins. The comminuted air-dry petals of Hibiscus cannabinus var. simplex collected in the middle of August (1 kg) were extracted first with chloroform and then, after the elimination of traces of chloroform, three times with methanol containing 1% of hydrochloric acid (at room temperature).

The methanolic extracts were combined and concentrated under vacuum at 30–35°C in an atmosphere of nitrogen to small volume (about 1.5 liter).

The concentrated extract was left in the refrigerator, the precipitate that deposited was filtered off, and the filtrate was treated three times with an excess of dry peroxide-free ether. The resulting precipitate of anthocyanins was separated by filtration, washed with ether and acetone, and dried rapidly under vacuum.

To free them from flavonols, the combined anthocyanins were dissolved in butan-1-ol–acetic acid–water (4 : 1 : 5) (system 2). The solution was passed through a column of cellulose powder (60 × 4.5 cm) and eluted with the same solvent. After concentration, the eluate was treated with a fivefold amount of petroleum ether (bp 40–70°C). The resulting precipitate was washed with petroleum ether and with acetone and was dried under vacuum. This gave 38 g (yield 3.8% of the air-dry raw material) of anthocyanins having a cherry-red/violet color with a golden tinge.

Preparation of Cannabinin. A solution of 2 g of the combined anthocyanin glycosides in system 1 was passed through a column of cellulose powder and was eluted with the same solvent until the colored zones of the anthocyanins were sharply separated. The separated zones were cut out and eluted with 0.01% hydrochloric acid in methanol. A methanolic eluate containing the anthocyanin with R_f 0.38 [water–acetic acid–hydrochloric acid (82 : 15 : 3), system 3] was obtained from the lower zone of the column; it was evaporated under vacuum at 30–35°C in an atmosphere of nitrogen until the methanol had been eliminated. Recrystallization of the residue from ethanol containing 1% of hydrochloric acid and drying the resulting microcrystalline powder over phosphorus pentoxide yielded 1.43 g of cannabinin $C_{26}H_{29}O_{16}Cl \cdot 4H_2O$ with mp 164–166°C (decomp.). Yield 4.15% (of the air-dry weight of the raw material), R_f 0.38 (system 3) and 0.14 (system 2); λ_{max} 531 nm (0.01% HCl in methanol).

Acid Hydrolysis of Cannabinin. A mixture of 0.1 g of the anthocyanin and 10 ml of 7% hydrochloric acid in methanol was heated in an atmosphere of nitrogen at 70–75°C for 1 h. Then, after concentration, the hydrolyzate was treated with 15 ml of water, the aglycone was extracted with isoamyl alcohol, the extract was concentrated in vacuum at 35–36°C in an atmosphere of nitrogen, and the anthocyanidin was precipitated with petroleum ether. The precipitate was filtered off, washed with petroleum ether, and recrystallized from 0.5 N hydrochloric acid in ethanol. This gave 0.055 g of delphinidin $C_{15}H_{11}O_7Cl \cdot 1\frac{1}{2}H_2O$, dark violet, mp 300°C (decomp.), λ_{max} 545 nm (0.1% HCl in methanol), with the addition of aluminum chloride 568 nm.

The aqueous part of the hydrolyzate after the extraction of the delphinidin was treated with activated carbon, and the filtrate was neutralized with saturated baryta solution and chromatographed on paper in two solvent systems: system 1 and system 4 [ethyl acetate–pyridine–water (2 : 1 : 2)]. To determine the sugars, one of the chromatograms was treated with a solution of aniline phthalate and the other with a mixture of an alcoholic solution of salicylic acid and o-toluidine. Glucose and xylose were found in the hydrolyzate.

Enzymatic Hydrolysis of Cannabin. A solution of 0.025 g of the anthocyanin in 0.4 ml of ethanol was diluted with water to 2 ml. To this solution was added 0.01 g of the enzyme of the fungus Aspergillus oryzae, and the mixture was left in the thermostat at 36°C for 36 h. The hydrolyzate, after being treated with activated carbon, was chromatographed in systems 1 and 3; two spots were found corresponding to glucose and xylose.

Oxidation of Cannabinin with Hydrogen Peroxide. A solution of 0.05 g of the glycoside in 2 ml of methanol was treated with 1 ml of ammonia and 0.6 ml of 30% hydrogen peroxide, and the mixture was left at room temperature for 4 h. Then 0.01 g of freshly prepared lead sulfide was added to decompose the excess of hydrogen peroxide. The excess of lead sulfide was filtered off and washed with water, and the filtrate was subjected to ammonolysis with 0.5 ml of ammonia (sp. gr. 0.88) and was heated in the water bath for 5-6 min. The degradation products were analyzed by paper chromatography in system 1 together with markers – glucose and xylose. The sugar shown up on the chromatogram had a R_f value corresponding to neither of these monoses. The spot was then treated with o-toluidine salicylate and heated at 100-105°C. A brown spot with R_f 0.17 (system 1), which is characteristic for disaccharides, was formed.

Acid Hydrolysis of the Bioside. A solution of the sugar obtained after the oxidative degradation of the cannabinin was treated with 2 ml of 5% hydrochloric acid solution and heated in the water bath for 15 min. Chromatography of the hydrolyzate in systems 2 and 4 showed the presence of glucose and xylose.

Isolation of Myrtillin. The feebly colored upper anthocyanin zone with R_f 0.12 (system 2) obtained after the separation of the combined anthocyanins in the isolation of the cannabinin was eluted with methanol containing 0.01% of hydrochloric acid. The methanolic eluate was distilled in vacuum in a current of nitrogen to dryness, and the residue was recrystallized from 0.3 N hydrochloric acid in ethanol. This gave 0.31 g (yield 0.89% of the air-dry raw material) of an anthocyanin $C_{21}H_{21}O_{12}Cl \cdot H_2O$ with mp 182-182°C (decomp.), R_f 0.12 (system 2) and 0.13 (system 1), λ_{max} 535 nm and with the additive 571 nm.

Acid Hydrolysis of Myrtillin. A solution of 0.08 g of the glycoside in 10 ml of 3.5% hydrochloric acid in methanol was heated in the water bath for 1 h. The hydrolyzate was evaporated to small volume and treated with 10 ml of water, and the aglycone was extracted with isoamyl alcohol. The extract was concentrated in vacuum at 35°C in an atmosphere of nitrogen, the anthocyanidin was precipitated with petroleum ether, and the precipitate was separated off, washed with petroleum ether, and recrystallized from 0.3 N hydrochloric acid in ethanol. This gave 0.04 g of delphinidin $C_{15}H_{11}O_7Cl \cdot 1\frac{1}{2} H_2O$ with mp above 300°C (decomp.), λ_{max} 545 nm, with the addition of aluminum chloride, 568 nm.

The aqueous part of the hydrolyzate after the extraction of the aglycone was treated with activated carbon, and the filtrate was evaporated to small volume and chromatographed on paper in systems 1 and 3; it was thereby shown to contain glucose.

Enzymatic Hydrolysis of Myrtillin. A solution of 0.02 g of the anthocyanin in 0.3 ml of ethanol was diluted with water to 2 ml. To this solution was added 0.01 g of the enzyme of A. oryzae, and the mixture was left in the thermostat at 36°C for 36 h. Chromatography of the hydrolyzate in systems 1 and 3 showed the presence of glucose.

Oxidation of Myrtillin with Hydrogen Peroxide. The products of oxidative degradation were analyzed by paper chromatography in systems 1 and 3 together with markers – monosaccharides (glucose, xylose, and galactose). The chromatogram revealed a spot corresponding to glucose.

Alkaline Cleavage of Delphinidin. A solution of the aglycone (0.06 g) in 6 ml of 15% baryta solution was heated in the water bath in an atmosphere of nitrogen for 45 min. The reaction mixture was acidified with sulfuric acid, and the cleavage products were extracted with diethyl ether. After concentration, the ethereal extract was chromatographed on paper in system 1 and system 5 [butan-1-ol–benzene–acetic acid–water (2 : 10 : 2 : 1)]. Revealing agents: a 1% solution of vanillin in concentrated hydrochloric acid, and a mixture of equal volumes of 1% solutions of ferric chloride and potassium ferricyanide.

Phloroglucinol and gallic acid were found in the alkaline cleavage products.

CONCLUSIONS

Two anthocyanin glycosides have been isolated from the flowers of Hibiscus cannabinus var. simplex. One of them is a new glycoside, which has been called cannabinin, and the second is the known glycoside myrtillin, this being the first time that it has been isolated from the flowers of Kenaf hemp.

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