PROTOLYTIC PROPERTIES OF FLAVONOLS FROM PEA CHLOROPLASTS

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Using the buffer capacity method, a study has been made of the protolytic properties of three flavonoids isolated from pea chloroplasts: kaempferol 3-D-triglucoside (KG), kaempferol 3-D-triglucoside p-coumarate (KGC), and quercetin 3-D-triglucoside p-coumarate (QGC). It has been shown that the curves of the buffer capacities of solutions of the flavonoid investigated have from two to four peaks of dissociation constants. When the substances participate in photobiochemical reactions, they are capable of manifesting protonophoric properties.

Continuing a study of the physicochemical properties of phenolic glycosides isolated from pea chloroplasts, we have investigated the protolytic properties of three flavonoids — kaempferol 3-D-triglucoside (KG), kaempferol 3-D-triglucoside p-coumarate (KGC), and quercetin 3-D-triglucoside p-coumarate (QGC) by the buffer capacity method [1]. The overall dependences of the buffer capacity were apportioned to the one-proton curves for determining the nominal microdissociation constants of the groups (pK_i) and the concentrations of these groups in solution (C_i).

The total buffer curves of KG, KGC, and QGC in the pH range of 6-10 at an ionic strength of the solution of 0.01 M KCl at a temperature of 20°C each have two well-defined maxima. For KG, the β-curve is distributed over two one-proton peaks of equal intensity $C_1 = C_2 = C_{reagent}$ with $pK_1 = 7.4$ and $pK_2 = 9.0$ (Fig. 1). The introduction of p-coumaric acid into the molecule does not change pK_1 and pK_2, which is explained by the screening effect of the three glucose molecules, but the intensity $C_1 < C_2 = C_{reagent}$. Apart from the decrease in C_1, the appearance of a peak at $pK_3 = 8.2$ with $C_3 = C_{reagent} - C_1$ is observed. This effect may indicate that part of the KGC molecule is present in the "folded form" as a consequence of

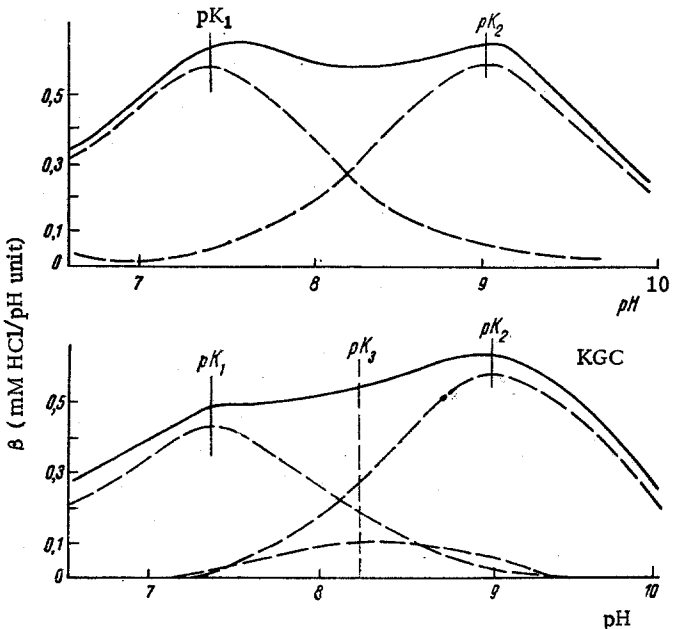

Fig. 1. Dependence of the buffer capacity of solutions of KG (0.6 mM) and of KGC (0.6 mM) on the pH of the medium in the presence of 10 mM KCl.

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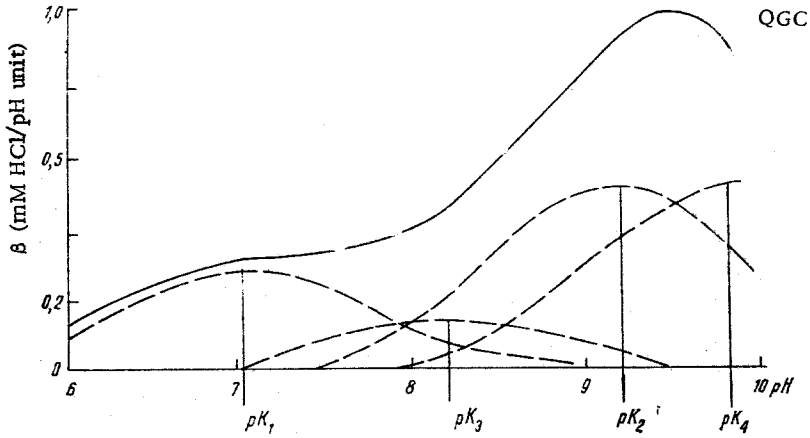

Fig. 2. Buffer spectrum of QGC (aqueous medium, 10 mM KCl, 0.6 mM QGC).

the formation of intramolecular hydrogen bonds. Consequently, the dissociation of one phenolic OH group (pK_3 = 8.2) is greatly inhibited and the dissociation of the OH group with pK_1 = 7.4 is weakened. The bond between the p-coumaric part of the molecule and the hydroxyls of the main radical breaks down at pH 8, since $C_2 = C_{reagent}$ and pK_2 of KGC coincides with pK_2 of KG.

In KGC and KG, pK_1 and pK_2 are identical, but the first concentration coefficient of KGC is less than unity and the curve has an additional peak with a pK value of 8.2. The presence of such a peak with no change in the value of the second concentration coefficient permits us to conclude that about 30% of the KGC is present in solution in a form in which the glucosyl p-coumarate part of the molecule is located close to ionogenic groups, which substantially inhibits the dissociation of one proton.

It is impossible to resolve the β-curve of QGC because of the possible appearance of the hydrolysis products in solution (Fig. 2). The following series of peaks can be isolated with the greatest confidence pK_1, 7.0; pK_2, 9.2; pK_3, 8.2; and pK_4, 9.8; as in the case of KGC. The concentration of OK groups corresponding to the peaks from the more acid pH region is less than the concentration of the reagent QGC in solution, while the alkaline peaks with pK 9.2 and 9.8 have $C_2 = C_4 = C_{reagent}$. This also shows the formation of intramolecular hydrogen bonds between the glucosyl p-coumarate and the main parts of the flavonol molecules.

Thus, our earlier suggestions that flavonol triglucosides possess protonophoric functions are confirmed by their protolytic properties [2, 3]. Consequently, it may be considered that at a pH of the photochemical reaction of 7.8, the substances have two degrees of dissociation as a minimum, and this permits us to consider that a flavonoid is capable of transferring two protons through the thylakoid membrane. In connection with these facts, a feature of quercetin and kaempferol derivatives as uncouplers of photophosphorylation consists in their polyprotonophoric capacity and expands their possibilities as substances with a regulatory function.

EXPERIMENTAL

The buffer curves in Figs. 1 and 2 were obtained with the aid of the manual differentiation of the potentiometric titration curve. The tangent to the experimental β(pH) curve was constructed and its slope in mM/pH unit was determined. In our experiments, the β scale was given by the rate of addition of titrant by a micropump. The background curve of the medium was determined similarly. The buffer curves of the magnets shown in the figures were plotted as the difference $\beta_{exp} - \beta_{medium}$. The rate of titration was selected in such a way that halving it did not change the shape of the curve (i.e., so as to fulfill the following condition: the rate of titration was much less than the rate of the conformational transition).

The investigation was performed in a thermostated call with a volume of 5 ml under a current of nitrogen at a temperature of 15°C.

SUMMARY

The protolytic properties of flavonoids isolated from pea chloroplasts have been studied. It has been shown that flavonol triglucosides have two to four dissociation constants, which permits them to exhibit protonophoric properties over a wide pH range.

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TERPENOIDS OF THE OLEORESIN OF THE LARCH GROWING IN KAMCHATKA

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The complete chemical composition of the oleoresin of the larch *Larix cajanderi* M. growing in Kamchatka has been studied. Fifty-four terpene compounds have been isolated and identified. It has been shown that among the monoterpenes the main constituents are α- and β-pinenes, their amounts being 66.3% and 18.3%, respectively. Among the sesquiterpene hydrocarbons the main components are δ-cadiene and γ-elemene (15 and 17%). Diterpenoids are represented mainly by bicyclic labdane compounds — epimanool (∿15%), larixylacetate (∿28%), and larixol (∿40%). In the acidic fraction of the oleoresin, isopimaric acid (40%) predominates.

Continuing a systematic investigation of the oleoresins of conifers, we have studied the terpenoids of the larch growing in Kamchatka. In the opinion of investigators, the central Kamchatkan populations of the larch belong to the species *Larix cajanderi* M. (Cajander's larch) and are considered as relict populations [1].

We have studied the complete chemical composition of the oleoresin of this species of larch. The oleoresin was treated by a method described previously [2] for separating the acidic components.

The neutral substances (50% of the weight of the oleoresin) were separated by adsorption chromatography into hydrocarbons and oxygen-containing compounds (16 and 34%, respectively).

The hydrocarbons consisting of a mixture of monoterpenes, sesquiterpenes, and diterpenes, were subjected to fractionation, which yielded 65% of monoterpenes, 5% of sesquiterpenes, and 25% of diterpenoids.

The monoterpene hydrocarbons were analyzed by gas—liquid chromatography (GLC). The main components were α-piene (66.3%) and β-piene (18.3%).

In the sesquiterpene fraction, 13 hydrocarbons were found by adsorption chromatography and gas—liquid chromatography, the main ones being δ-cadiene (15%) and γ-elemene (12%). The individual sesquiterpenes were identified by their IR and PMR spectra (see the experimental part).

In the diterpenoid fraction, hydrocarbons and aldehydes were detected. Of the hydrocarbons, isopimaradiene, abietadiene, and dehydroabietadiene were identified, their amounts being very small.

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