CATECHIN GALLATES OF RHEUM MAXIMOVICZII A. LOS.

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In a study of the polyphenol composition of the roots of Rheum maximoviczii A. Los (mountain rhubarb) gathered in the Tyan'-Shan mountains we have found thirteen polyhydroxyflavanols, of which nine are catechins [1]. The separation of these monomeric polyphenols from their polymerization products was effected by chromatographing an aqueous extract of the roots on rawhide powder. The polyhydroxyflavanols were selectively desorbed with ether and ethyl acetate. The total catechins were separated by partition chromatography on silica gel. Two catechins were identified as (+)-catechin and (−)-epicatechin [2].

The present paper gives results on the identification and quantitative determination of two isomers of catechin gallate. These flavonoids were desorbed from the column of rawhide powder with ether. On chromatography on silica gel, catechin gallate (I) formed a zone with R_1 0.70 and R_2 0.54, and catechin gallate (II) a zone with R_1 0.84 and R_2 0.68.

Chromatography of catechin gallate (1) on "Leningrad rapid" paper in 2% acetic acid gave R_f 0.24, and in butanol-acetic acid-water (40:12.5:29) (BAW) R_f 0.76. Such a position on chromatograms and the qualitative reactions are characteristic for catechin gallate. From its formation on the enzymatic hydrolysis with tannase of (−)-epicatechin and gallic acid, this flavonoid was identified as (−)-epicatechin gallate. The identity was confirmed by its physical constants, which corresponded to literature data [3] for (−)-epicatechin gallate isolated from the leaves of green tea and also by the quantitative analysis of the catechin itself and its acetyl derivative.

The catechin gallate (II) was separated from resin and accompanying catechin by chromatography on Kapron and cellulose. After purification on cellulose, the catechin under investigation gave, in two-dimensional chromatograms, a single spot with R_f 0.09 in 2% acetic acid, and one with R_f 0.70 in BAW. After desorption from cellulose, two spots appeared on the chromatograms. The R_f values in BAW of the two spots were the same, while in 2% acetic acid they differed from one another with values of 0.06 and 0.12. It is known that this situation is characteristic for optical stereoisomers of the catechin [4] and cellulose, being an optically active adsorbent, is capable of separating catechin racemates [5].

On heating with alkali, the flavonoid was cleaved with the formation of phloroglucinol and protocatechuic and gallic acids. The latter is split off by enzymatic hydrolysis, a catechin being produced sterically analogous to (+)-catechin. The absence of optical activity and separation into two stereoisomers by interaction with cellulose showed that this compound was (±)-catechin gallate. This catechin has not previously been isolated from plants.

A quantitative determination of the catechins was carried out by densitometry directly on the paper after separation by two-dimensional chromatography in the systems of solvents mentioned above and detection with silver nitrate. The measurements of the maximum optical density of the spot coloration was carried out with a "Magnefot 2" photoelectric densitometer. The concentrations were calculated from calibration curves constructed for pure catechins. The roots collected in May were found to contain 0.16% of (−)-epicatechin gallate and 0.026% of (±)-catechin gallate on the weight of the absolutely dry roots, and the roots prepared in August were found to contain 0.09% of (−)-epicatechin gallate and 0.018% of (±)-catechin gallate.

Experimental

Purification of catechin gallate (I). The substance was purified by partition chromatography on grade KSK silica gel treated by the method described previously [1], using water as the stationary liquid phase and ether as the mobile liquid phase in an atmosphere of nitrogen. The ratio of the mixture of substances to be separated to the silica gel was 1:150. The separation was followed by means of qualitative reactions of the eluate with vanillin in concentrated hydrochloric acid and with ferric ammonium alum. The dry residue after the ether had been evaporated off was crystallized from water, mp 252°C; $[\alpha]_D^{20}$ −170° (c 0.35; ethanol); λ_{max} 280 mμ.

Found, %: C 59.40; H 4.36. Calculated for $C_{22}H_{18}O_{10}$, %: C 59.72; H 4.04.

Acetylated catechin gallate (I). A solution of 150 mg of the substance in 3 ml of acetic anhydride was heated for 5 hr in a boiling water bath in the presence of 500 mg of anhydrous sodium acetate, and then for a further 1 hr with the reaction mixture gently boiling. The reaction product was poured into 30 ml of water. The mass formed was crystallized from aqueous methanol, mp 119°C, $[\alpha]_D^{20}$ −94° (c 0.38; benzene). The physical constants and elementary composition correspond to a heptaacetate of (−)-epicatechin gallate.

Found, %: C 58.41; H 4.60. Calculated for $C_{36}H_{32}O_{17}$, %: C 58.69; H 4.34.

Separation of catechin gallate (II). The substance was separated from accompanying catechin and resins by chromatography on Kapron from aqueous solution. The ratio of the total substances to be separated to the adsorbent was 1:5. Desorption with ether gave 24 40-ml fractions. The catechin gallate under investigation was present in fractions 8-12. The ether was distilled off in an atmosphere of nitrogen at a bath temperature not exceeding 40°C. The final purification of the catechin gallate from resins was achieved by chromatography on cellulose using water as eluant. The resins were retained in the upper part of the column. The aqueous eluate of catechin gallate was concentrated by lyophilization. The dry residue was recrystallized from water. This gave an amorphous powder with mp 195-197°C (decomp) and λ_{max} 280 mμ; the substance was optically inactive (c 0.76; ethanol), and was readily soluble in water, alcohols, and ether, and insoluble in benzene and chloroform. On chromatograms it had a dark purple fluorescence in UV light and gave a deep blue coloration with iron salts and a bright pink coloration with vanillin in concentrated hydrochloric acid.

Enzymatic hydrolysis of the catechin gallates. A solution of 20 mg of the substance in 2 ml of water was treated with 10 mg of tannase obtained from the fruit of myrobalan by Freudenberg's method [6]. The mixture was kept in a thermostat at 34°C for seven days. The course of the hydrolysis was checked by two-dimensional paper chromatography of samples of the hydrolyzate. As a result of the hydrolysis, the spot of the initial flavonoid disappeared and two new spots appeared, one of which corresponded to a reference spot of gallic acid (R_f 0.35 in 2% acetic acid and R_f 0.67 in BAW; deep blue fluorescence in UV light, deep blue coloration with ferric ammonium alum). In addition to gallic acid the hydrolysis of the catechin gallate (I) gave a clear spot corresponding to (−)-epicatechin (R_f 0.30 in 2% acetic acid and R_f 0.58 in BAW), while the hydrolysis of the catechin gallate (II) gave a spot corresponding to (+)-catechin (R_f 0.33 in 2% acetic acid and R_f 0.66 in BAW).

Alkaline degradation of the catechin gallate (II). In an atmosphere of nitrogen, 170 mg of the substance was heated in 6 ml of 50% caustic potash for 30 min at 150°C. After cooling, the alkali was neutralized with 12 ml of 25% sulfuric acid. The ethereal extract of the reaction products was chromatographed on paper. Three spots were found, corresponding to phloroglucinol (R_f 0.56 in 2% acetic acid and R_f 0.77 in BAW; orange-red coloration with vanillin in hydrochloric acid, pale yellow coloration with diazotized p-nitroaniline), gallic acid (R_f 0.35 in 2% acetic acid and R_f 0.67 in BAW; deep blue coloration with iron salts) and protocatechuic acid (R_f 0.56 in 2% acetic acid and R_f 0.84 in BAW; light purple fluorescence in UV light, green coloration with ferric ammonium alum, orange coloration with diazotized p-nitroaniline). Their identities were confirmed by comparison with reference samples.

Summary

The roots of Rheum maximoviczii A. Los. contain two catechin gallates: (−)-epicatechin gallate, previously isolated from the leaves of green tea and (±)-catechin gallate found in this plant for the first time. The amount of catechin gallates in the roots is lower in the autumn than in the spring.

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