FORMATION OF PHENOLIC COMPOUNDS IN COTTON SPROUTS

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In previous papers [1-4] an account has been given of the composition of the tanning substances present in various organs of the cotton plant and the isolation of catechins from some of its organs.

The chemical composition of the polyphenols has enabled us to elucidate their formation and transformation mechanisms during the growth of the plants as well as the relationship of the catechins to the anthocyanidins and the flavonols in the cotton plant.

In this study we used: Leaves and rootlets of 3-, 5-, 7-, 10-, and 15-day sprouts grown in the dark and in the light; the leaves and the bark of roots and stems in the incipient budding period; 15-day boll carpels; and the seeds of cotton plants grown under natural conditions.

As can be seen from the chromatograms given (Fig. 1), the simplest component (+)-catechin is formed from the very first days of growth of the seeds of the cotton plant in the cotyledons and rootlets both in the light and in the absence of light (etiolated sprouts). From seven days' growth, (±)-gallocatechin is found in the cotyledons and rootlets. In addition, an anthocyan with R_f 0.23 and small amounts of (−)-epigallocatechin, (±)-catechin, and (−)-epicatechin are formed in the rootlets.

As the plant grows in the light, in addition to (+)-catechin and (±)-gallocatechin, the flavonol quercetin accumulates in the leaves (Fig. 2), while no deposition of flavonols is observed in the leaves of etiolated sprouts.

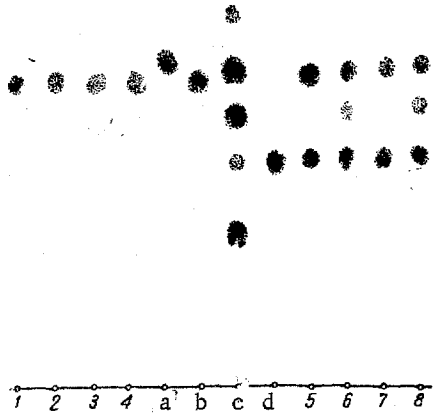


Fig. 1. Chromatograms of cotton plant polyphenols (3-day and 7-day sprouts). 1-4) 3-day sprouts, 5-8) 7-day sprouts: 1) Cotyledons; 2) rootlets; 3) etiolated cotyledons; 4) etiolated rootlets; a) quercetin; b) (+)-catechin; c) standard preparation of tea catechins; d) (+)-gallocatechin isolated from tea leaves; 5) cotyledons; 6) rootlets; 7) etiolated cotyledons; 8) etiolated rootlets.

When etiolated sprouts are illuminated, chlorophyll and flavonols form in the leaves, while catechins disappear. In the second, third, fifth, sixth, eighth, and ninth sympodial leaves under natural conditions, other flavonoids beside quercetin are formed in large amount, such as isoquercitrin and a flavonol with R_f 0.73. At the same time catechins and gallocatechins accumulate in other organs of the cotton plant (the bark of the roots, the stems, the boll carpels, and seeds) (Fig. 3).

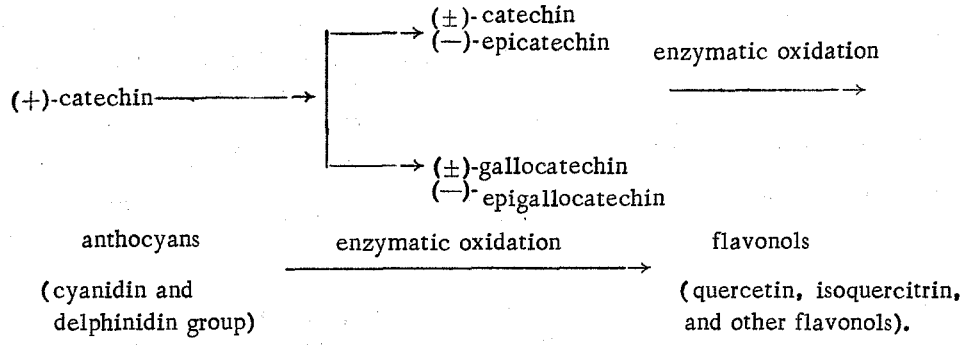
A study of the composition of the polyphenols in various organs of the cotton plant has shown that the whole complex of catechins contained in the cotton plant is formed in the bark of the roots and stems of the plant grown under natural conditions beginning from the period of budding, and in the boll carpels and seeds after 15 days of growth.

Like the catechins, the anthocyans accumulate in the cotton sprouts both in the light and in the dark. It can be seen from the literature information available at the present time that catechins [5] and anthocyans [6-7] can also form in the dark.

The information that we have obtained indicates that light is necessary for the formation of flavonols in the leaves of the cotton plant.

The results of the experiments given above confirm that the appearance of catechins and anthocyans takes place even in the dark but light energy accelerates their accumulation, while the formation of flavonols is associated with photosynthesis, as has been observed by other workers [5-8]. Since the formation of flavonols in the leaves is accompanied by the disappearance of the catechins both when etiolated sprouts are illuminated and under natural conditions, it may be assumed that the simplest catechins [(+)-catechin and (−)-epicatechin] are capable of being converted by illumination into the most widespread flavonol, quercetin.

On the basis of the experimental and literature material presented [6], it is assumed that the formation of the phenolic compounds of the cotton plant takes place in accordance with the following scheme:



Experimental

The seeds of cotton plant of variety 108-F were treated with 50% sulfuric acid to eliminate fuzz and were washed with water to neutrality.

The seeds previously wetted for 24 hours were germinated in a crystallizing dish. The sprouts appearing were transplanted into another crystallizing dish and were covered with water in such a way that the rootlets were only just immersed in the water. Then the crystallizing dish and contents were placed in a thermostat at a constant temperature (30°), and growth was allowed to take place both in the light and in the dark. A temperature of 30° was the optimum for the growth of the seeds [9], since at this temperature the development of the rootlet, the bud and the hypocotyl takes place best.

Cotton seeds were also grown in a field where shoots appeared several days later than in the laboratory.

The formation and transformation of the individual phenols in the cotton sprouts and other organs of the further growth of this plant were studied by means of paper chromatography [10].

The sprouts were separated into leaves and rootlets and were then separately triturated with methanol in a porcelain mortar until the phenolic compounds had been extracted completely (test with a 1% solution of vanillin in concentrated hydrochloric acid and ferric chloride).

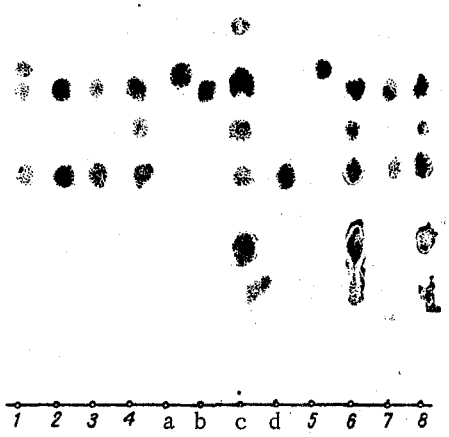


Fig. 2. Chromatograms of the polyphenols of 10-day and 15-day cotton sprouts: 1)-4) 10-day and 5)-8) 15-day cotton sprouts (other symbols as for Fig. 1).

The filtered solutions were combined and concentrated in vacuum and the syrupy extract was treated with chloroform to eliminate the gossypol, resins, and oily substances, after which the residue was dissolved in alcohol. This

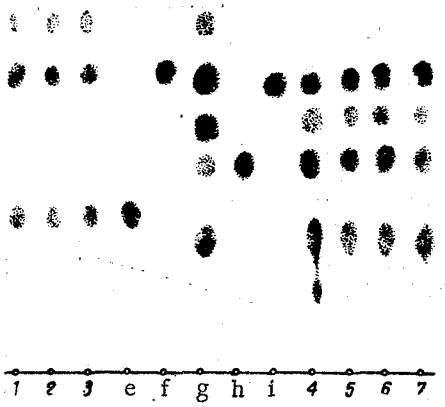


Fig. 3. Chromatograms of the flavonols and catechins in the leaves, bark, roots, stems and boll carpel, and seeds of the cotton plant. 1) From 2nd and 3rd; 2) from 5th and 6th, and 3) from 8th and 9th sympodial leaves; e) isoquercitrin; f) quercetin; g) standard preparations of tea catechins; h) (±)-gallocatechin; i) (+)-catechin; 4) bark of the roots in the period of incipient budding; 5) bark of the stems in the same period; 6) 15-day boll carpels; 7) seeds (seed hulls) of the 15-day boll.

solution was used for chromatography. Solvent system: butan-1-ol – acetic acid – water (40 : 12 : 28). Detecting agent: 1% solution of vanillin in concentrated hydrochloric acid; 1% solution of ferric chloride and potassium ferricyanide (1 : 1). In addition, the chromatogram was examined in UV light after detection. Alcoholic solutions of (+)-catechin [8], quercetin, isoquercitrin [6], (±)-gallocatechin, and a standard preparation of the combined catechins of tea leaves were used as markers for the identification of individual polyphenols of the cotton plant.

The standard preparation of the combined catechins was given to us by M. N. Zaprometov.

Summary

The simplest component of the catechin complex, (+)-catechin, is formed in the cotyledons and rootlets from the very first days of the development of cotton seed. This component accumulates both in the light and in the dark. As the plant grows, catechins gradually disappear from the leaves of the cotton plant and flavonols remain. Catechins and gallocatechins are formed in the boll carpels and the bark of the roots and stems.

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