

Studies on the Benzotropolone Pigment formed from (+)-Catechin and Gallic acid

Part I. Synthesis of the Benzotropolone Pigment from (+)-Catechin and Gallic acid

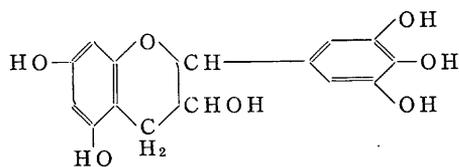
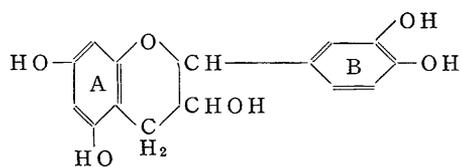
by Hiroaki HORIKAWA

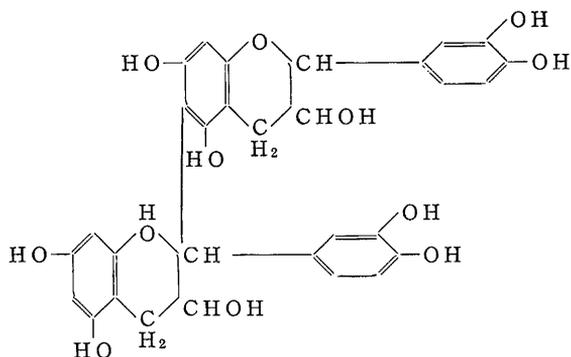
The present work is one of the studies carried out to elucidate a reddish pigment in black tea. A compound having a resemblance to a black tea pigment was synthesized from (+)-catechin and gallic acid. From a consideration of its chemical characters, it was supposed to be a troponoid compound having a 3', 4'-dihydroxy benzotropolone nucleus as suggested by a tentative structural formula (XII).

Introduction

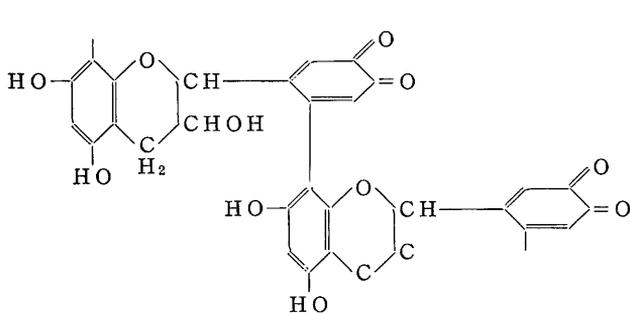
In black tea manufacture, the most important components are catechins, which effect on water soluble reddish color, taste and etc.. It has been known that catechins are oxidized to a characteristic reddish color by the polyphenol oxidase occurring in green tea leaf in process of manufacture, but its oxidation mechanism and its chemical structure are not yet clear completely. Recently, Y. Takino et al., have elucidated about them in details.

For a long time, it has been said that catechins are oxidized to the flobaphen which is dark brown and insoluble in water. Freudenberg et al.,¹⁾ showed that by oxidation with mineral acids, catechin (I) formed a dimer of structure (II) having a tanning action;

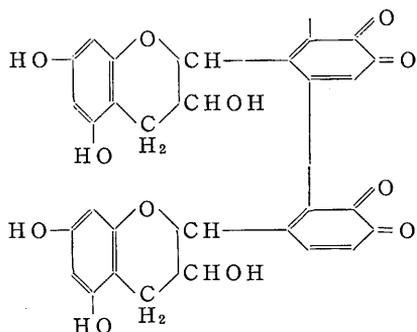




(III) Catechin dimer



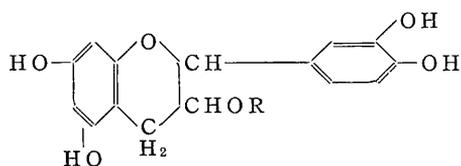
(IV) Head to tail polymer



(V) Tail to tail polymer

Harthway et al.,²⁹ studied on the auto-oxidation of catechin (I) and showed that it was oxidized to a polymer of structure (IV) by the reaction called head to tail. He also studied on the oak-bark tannin and supposed that galocatechin (II) was oxidized to polymerize tail to tail, by the links of C-2' and C-6' successively (V).

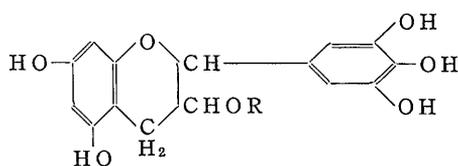
Tea leaf catechins consist of some components, the major of which are as follows ;



Catechol type catechin

(VI) R: H Epicachin

(VIII) R: galloyl Epicatechin gallate



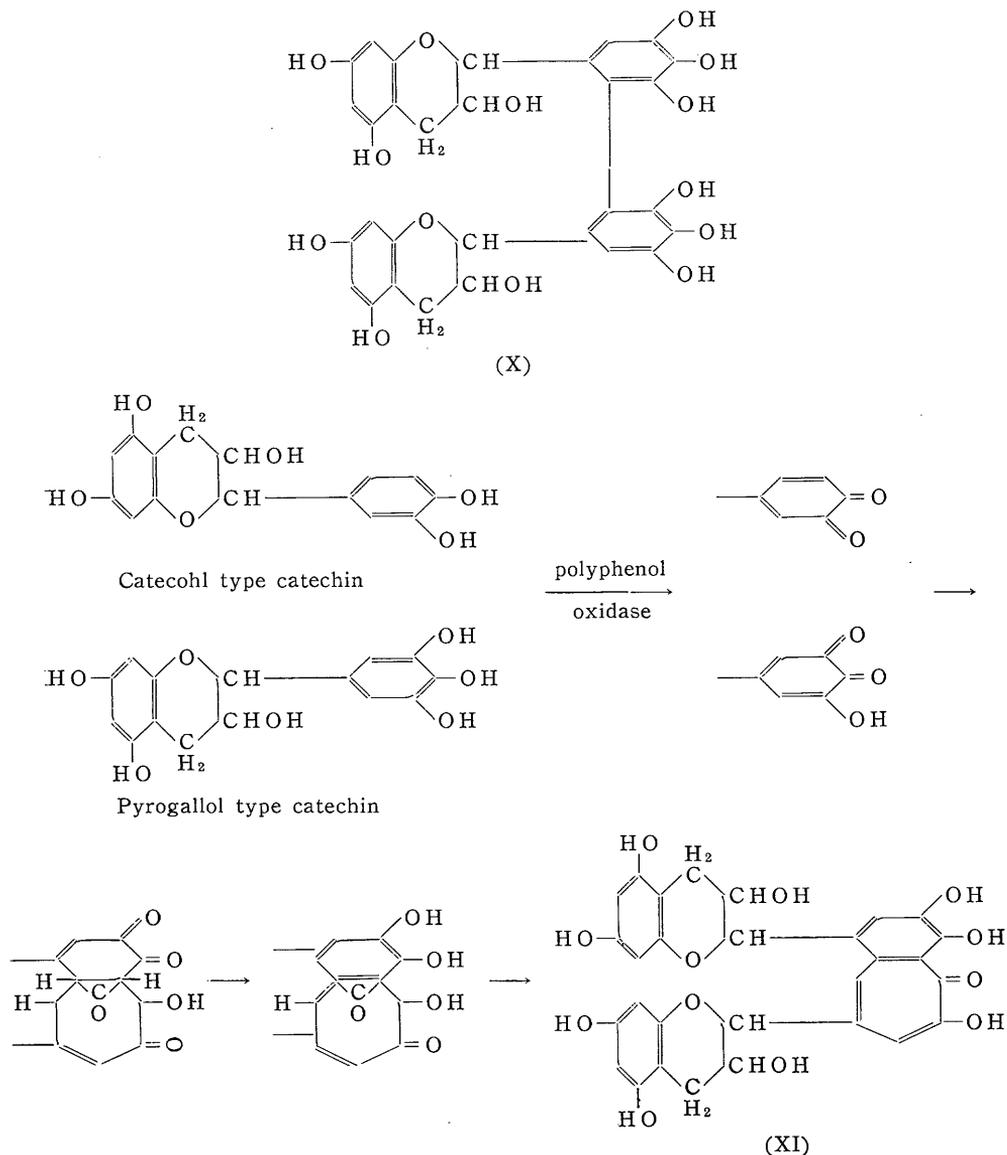
Pyrogallol type catechin

(VII) R: H Epigallocatechin

(IX) R: galloyl Epigallocatechin gallate

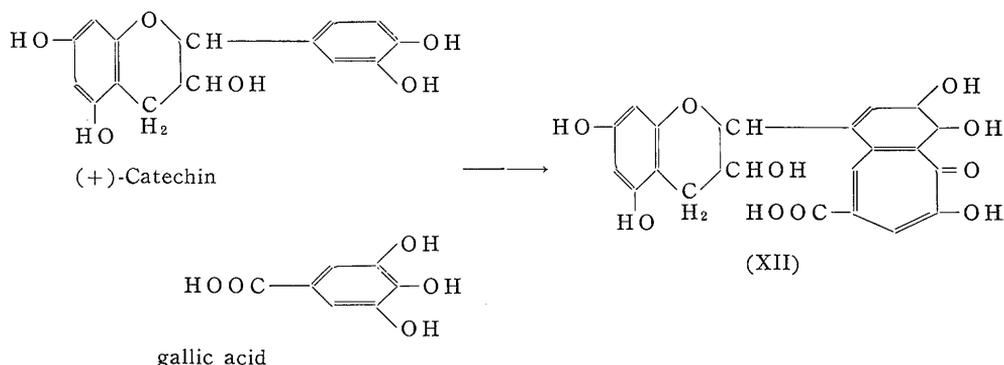
Roberts et al.,³⁰ presumed that a mixture of catechin (VII) and (IX), or two moles catechin (IX), these were pyrogallol type only, were oxidized to form a dimer compound and it was water soluble reddish color pigment of black tea. On the other hand, Y. Takino et al.,⁴⁰ carried out the oxidation of catechins of pyrogallol type only and proved that

ethylgallate was oxidized to diethyl-4, 4', 5, 5', 6, 6'-hexahydroxydiphnate and myricitrin to 2', 2'''-bimyricitrin, that is to say, by the oxidation of pyrogallol type catechin only, a colorless catechin dimer (X) was formed. Continually, he showed a reddish pigment of black tea was formed by coupling pyrogallol type catechin with catechol type and suggested its oxidation mechanism and a tentative structural formula as follows;



It is characteristic that there is dihydroxybenzotropolone nucleus in molecule. He supposed that a reddish color of black tea was attributed to this nucleus. As regards the chemical structure of this pigment, proofs have been getting by means of NMR, mass spectra and

etc.. But it is very difficult to get this pigment from black tea purely and enough amount.. So, author attempted to synthesize the compound which has the same dihydroxybenzotropolone nucleus and is able to form from obtainable materials easily. This purpose was achieved by treating a mixture containing (+)-catechin and gallic acid with potassium ferricyanide reagent. Its compound seems to be formed by condensing via quinon as well as above mentioned scheme;



Experimental

I. Oxidation of a Mixture of (+)-Catechin and Gallic acid

1) Preparation of (+)-Catechin

Gambir chatechu was refluxed in anhydrous ethyl acetate for an hour and then, the ethyl acetate extracts were concentrated under reduced pressure. On cooling the concentrate, crude catechin was crystallized out. It was recrystallized from hot water and used as (+)-catechin sample.

2) Preparation of an Oxidizing Reagent

Equal volume, each of aqueous potassium ferricyanide (3.08 g in 10 ml) and aqueous sodium hydrogen carbonate (0.78 g in 10 ml) was used.

3) Performance of Oxidation and Test for its Products

Eighteen milliliters of aqueous potassium ferricyanide were added to 80 ml of aqueous solution containing (+)-catechin (2 g) and gallic acid (0.5 g), to which 18 ml of aqueous sodium hydrogen carbonate were added dropwise with stirring and ice-cooling about 5°C. As the reaction proceeded, the color of solution turned into a reddish orange and soon a reddish dark products separated. The chromatogram of this reaction solution showed that as the reaction proceeded, (+)-catechin and gallic acid decreased and simultaneously spot I appeared and became more and more remarkable.

The paper (15×15 cm) was run first from left to right with 2% aqueous acetic acid and then, upwards with *n*-butanol-acetic acid-water (4:1:5 v/v). This spot turned into a characteristic blue color immediately, peculiar to phenolic hydroxy groups, on spraying a ferric alum reagent (mixture of equal volume of 0.5% aqueous ferric alum and 0.5%

aqueous ferric chloride), and pink on spraying an aqueous aluminium chloride. These properties coincided in those of benzotropolone. So, this product seemed to be the pigment sought for and therefore, a large scale oxidation was carried out to obtain enough amount of further studies.

II. Isolation and Purification of the Pigment (XII)

(+)-Catechin (16 g) and gallic acid (4 g) were dissolved in 700 ml of hot water, to which potassium ferricyanide solution (140 ml) were poured and then, cooled at 5°C.

One hundred and forty milliliters of sodium hydrogen carbonate solution were added to the above prepared solution dropwise in about thirty minutes, and then setting for ten minutes. In order to separate the resultant precipitates, the acidity of the solution was adjusted to pH 2~3 with dil. sulfuric acid. The precipitates were collected by filtration, washed with water to remove unreacted catechin and gallic acid and then, dissolved in anhydrous ethyl acetate. Insoluble matters were filtered off. The bright reddish pigment was obtained by concentration of the filtrate to dryness under reduced pressure. Recrystallization from anhydrous methanol yielded a reddish needle crystals (about 1 g), giving the same spot in Fig 1.

III. Properties of the Pigment (XII)

1) On spraying the ferric alum, turns blue immediately, peculiar to phenolic substances and pink with aqueous aluminium chloride. The crystal darkens at above 211°C: Values of elementary composition agree to the theoretical those of a tentative structural formula (XII): Calcd. for $C_{21}H_{16}O_{10}$: C, 58.88; H, 3.77%. Found: C, 58.45; H, 3.92%. Comparison of the ultraviolet spectra with those of 1',2'-dihydroxybenzotropolone 4-carboxylic acid which was prepared by the method Murakami et al.⁵⁾, are shown in Fig. 2 and 3. On considering both figures, it is expected obviously that the pigment (XII) has dihydroxybenzotropolone nucleus in molecule.

2) Preparation of Methyl Derivatives

i) Hexamethyl Derivative

Five hundred milligrams of the pigment (XII) were dissolved in anhydrous acetone (800 ml) and boiled slightly with anhydrous potassium carbonate (12 g) and dimethyl sulfate (5 ml) under reflux for ten hours. As the reaction proceeded, a reddish solution turned to yellow gradually. The resultant solution was filtered to remove potassium carbonate and

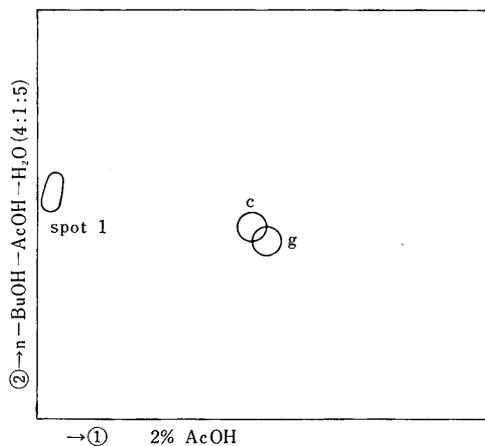


Fig. 1. Chromatogram of oxidation of a mixture of (+)-catechin and gallic acid.

g: gallic acid c: (+)-catechin
spot I: product

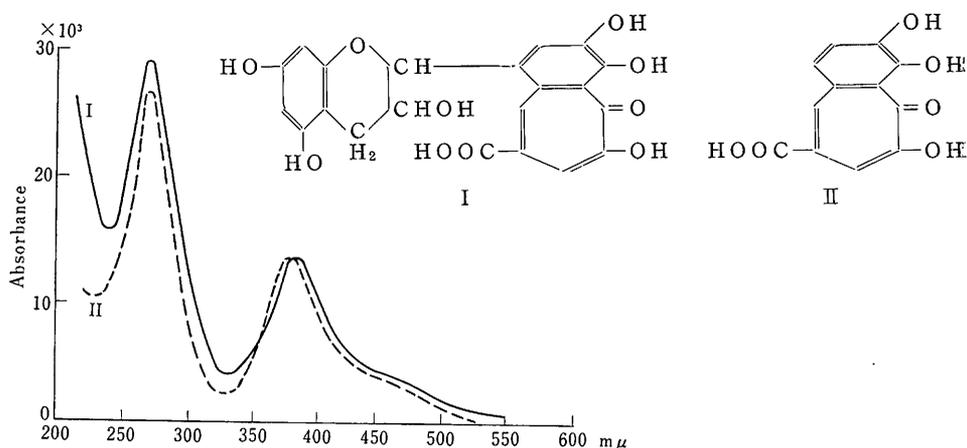


Fig. 2. Absorption Spectra of the Pigment (XII) and 1', 2'-Dihydroxybenzotropolone 4-carboxylic acid.

I: Pigment (XII). II: 1', 2'-Dihydroxybenzotropolone 4-carboxylic acid
Solvent: 50% aqueous methanol. Observed in 1-cm cells.

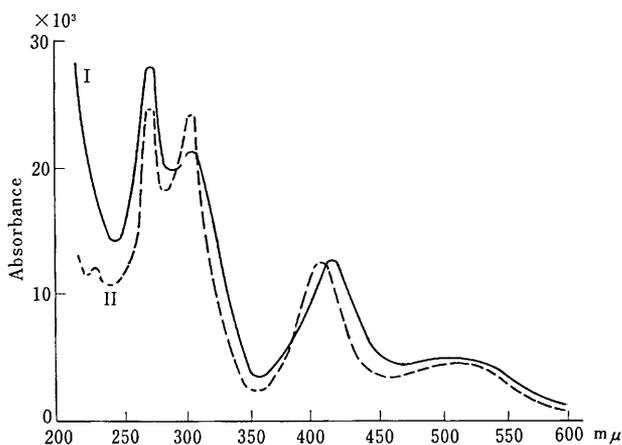
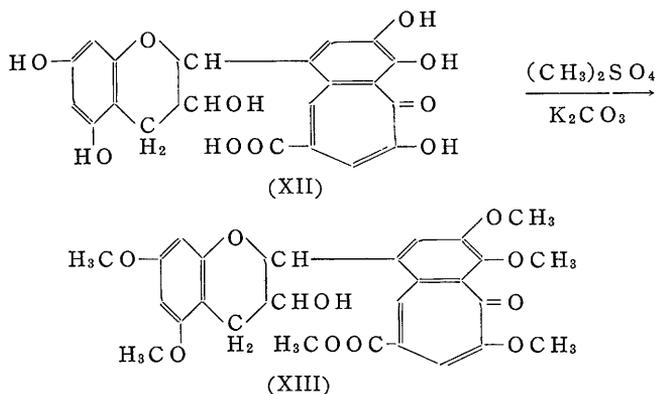


Fig. 3. Absorption Spectra of the Pigment (XII) and 1', 2'-Dihydroxybenzotropolone 4-carboxylic acid in 0.4% aqueous Aluminium chloride.

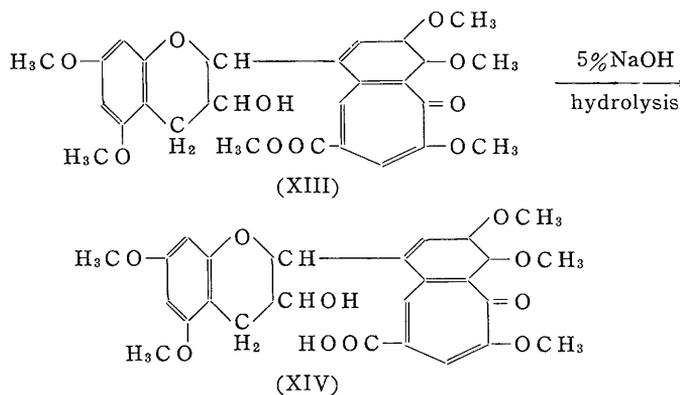
I: Pigment (XII), II: 1', 2'-Dihydroxybenzotropolone 4-carboxylic acid.
Observed in 1-cm cells.

then, the filtrate was concentrated to a dark brown solution containing excess dimethyl sulfate. On disintegrating excess dimethyl sulfate with 20% aqueous sodium hydroxide, the solution separated a yellowish solid gradually. The solid was collected, washed with water and dried. Recrystallization from 95% ethanol twice gave white yellow needles-crystals (XIII) 200 mg, m. p. 104~105°C. Anal. Calcd. for $C_{27}H_{28}O_{10}$: C, 63.27; H, 5.51%; CH_3O , 36.33%; Found, C, 63.08; H, 5.71%; CH_3O , 36.41%.



ii) Pentamethyl Derivative

A portion of hexamethyl derivative (100 mg) refluxed with 5% methanolic sodium hydroxide (5 ml) for half an hour to hydrolyze the ester linkage. After cooling and adjusting to pH 5.4, the resultant solution concentrated to separate a yellowish substance. This substance was collected, washed and dried. Recrystallization from glacial acetic acid gave white yellow needles crystals (XIV) 60 mg, m. p. 234~235.5 C (accompanied with decomposition). Anal. Calcd. for $C_{26}H_{26}O_{10}$: C, 62.64; H, 5.26; CH_3O , 31.12%: Found. C, 62.46; H, 5.34; CH_3O , 31.09%.



Summary

By oxidation of the mixture containing (+)-catechin and gallic acid, a reddish pigment was obtained. The ultraviolet and visible absorption spectra of it and its chelate compound with aluminium chloride are similar to those of 1', 2'-dihydroxybenzotropolone 4-carboxylic acid, extremely (Fig. 2 and 3). This fact means the possibility having dihydroxybenzotropolone nucleus in molecule of pigment (XII). Therefore, also in the case of reaction of (+)-catechin and gallic acid, the same reaction scheme as the formation of black tea pigment, may be formulated as shown page 2. The results of elementary analyses of the pigment (XII) and its methyl derivatives supports this suggestion.

Acknowledgements

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Reference

- 1) K. Freudenberg, J. H. Stocker and J. Porter. Chem. Ber., 90 957 (1957).
- 2) D. E. Hathway and J. W. T. Seakins. Biochem. J., 67 239 (1957).
D. E. Hathway. J. Chem. Soc., 1958 520.
- 3) E. A. H. Roberts, R. A. Cartwright and M. Oldschool. J. Sci. Food Agr., 8 72 (1952).
E. A. H. Roberts and M. Meyers. *ibid.*, 10 167, 176 (1959).
- 4) Y. Takino and H. Imagawa. Agr. Biol. Chem., 26 541 (1962), 27 319 (1963).
Y. Takino, H. Imagawa, Y. Aoki and T. Ozawa. *ibid.*, 27 562, 666 (1963).
Y. Takino, H. Imagawa, H. Horikawa and Y. Tanaka. *ibid.*, 28 64 (1964).
- 5) M. Murakami, K. Shzuki and E. Mishima. J. Chem. Soc. Japan, 75 620 (1954).