Reddish Orange Pigments of Black Tea Structures and Oxidative Formation from Catechins

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Tea leaves contain large quantities of catechin polyphenols, which undergo a series of chemical changes with the help of an oxidase during the manufacture of black tea and turn to reddish orange pigments (theaflavins) or brown pigments (thearubigins) characteristic of black tea. As the oxidative changes of catechins progress, strong astringency of them is modified and gradually disappears. Also it has been known that the coupled oxidation of polyphenols with amino acids results in the formation of volatile carbonyl compounds, which are responsible partly for flavor. Thus, catechins play an important role in developing liquor color of manufactured tea, and they also make contribution to the taste and aroma of tea infusions. Therefore chemical elucidation of the oxidative changes of catechins during the manufacturing process is considered to be a problem of significance not only in scientific respect but also for practical purpose.

Fresh tea leaves contain two types of catechins and their gallates as principal polyphenols, i.e. (−)-epicatechin(EC), (−)-epigallocatechin(EGC), (−)-epicatechin gallate (ECG) and (−)-epigallocatechin gallate (EGCG). The characteristic structural difference between these two types of catechins is the hydroxylation pattern of B-ring; EC has phenolic *o*-dihydroxyl groups, while EGC has phenolic *vic*-trihydroxyl groups. As these four principal catechins are contained together in fresh leaves and their oxidative changes taking place in the leaves during the manufacture seemed to be complicated, the author intended to elucidate the mechanism of these reactions analytically *in vitro*, carried out oxidations of individual catechins and related compounds as models, alone or in suitable combination of them, with insoluble tea oxidase and also with oxidizing reagents, and compared the properties of the reaction products with those of the constituents of black tea.

Oxidation of *vic*-trihydroxyphenolic catechins

In the oxidative reaction of catechins of this type with tea oxidase the formation of polyphenolic substances A_1 and A_2 from EGC, substance C from EGCG and substance B from the mixture of both catechins, respectively, was recognized[1] as also reported by Roberts et al.[2] They supposed the substances to be dimeric condensation products of each catechin, but their tentative formulas being devoid of enough experimental proof, the author intended to find evidence supporting their hypothesis. Noticeable common feature of the reactions visualized by the chromatographic analysis was that the position of the products on the paper chromatogram shifted in the parallel direction from those of the original catechins. The fact indicated that they were presumably formed in the same course of reaction and the chemical changes took place at the common *vic*-trihydroxyphenyl group in their molecules. In order to ascertain this assumption, ethyl gallate(EG) was incubated with oxidase as a key substance, and the principal oxidation product D, which showed the same chromatographic relationship as observed in the oxidation of the catechins, was obtained in slightly yellowish prisms, the structure of which was proved to be a dimer

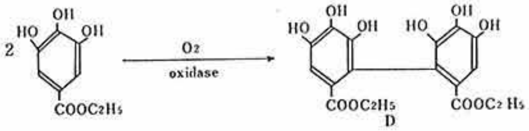

Fig. 1. Enzymic oxidation of ethyl gallate

of ethyl gallate, diethyl 4,4′, 5,5′, 6,6′-hexahydroxydiphenate[3] (Fig. 1).

Another valuable example for understanding the mechanism of the oxidation of catechins having a *vic*-trihydroxyphenyl group (pyrogallol type) was found in the study on the behavior of structurally related flavonoids during the tea manufacture. It was observed that myricetin-glycosides in fresh tea leaves decreased markedly during leaf-rolling and fermentation process. This fact suggests that they may be oxidized by the action of tea oxidase. As the aglycon myricetin has the identical carbon skeleton and hydroxylation pattern with those of EGC, the behavior of myricetin glycoside in the enzymic oxidation reaction is expected to present some reliable information for the oxidative changes of EGC and EGCG. Myricitrin (myricetin-3-rhamnoside) was used as a model substrate and incubated with tea oxidase. Myricitrin alone was not oxidized, but the addition of a substance containing *o*-dihydroxyphenyl group, such as (+)-catechin or catechol, caused rapid oxidation of myricitrin and resulted in the formation of crystalline substances G_1, G_2, and G_3. They also showed similar positional shift on the paper chromatogram, and the structure of G_3, one of the main products, was established to be a new dimeric flavonoid, 2′, 2‴-bimyricitrin[4] (Fig. 2).

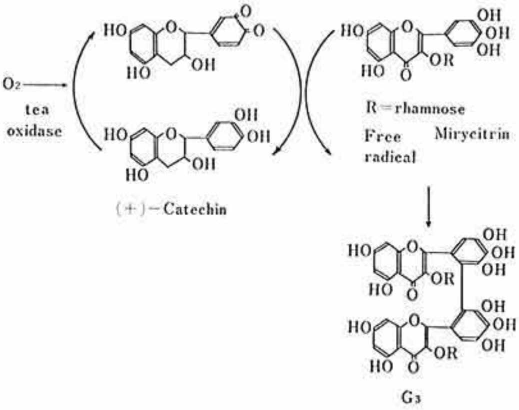

Fig. 2. Enzymic oxidation of myricitrin in the presence of (+)-catechin

The formation of biphenyl type dimer from ethyl gallate and myricetin glycoside by enzymic oxidation shows that this is the common type of reaction characteristic of *p*-substituted *vic*-trihydroxyphenol compounds. Further, considering the chromatographic common relationship between these phenolic compounds and their oxidation products, substances A_1, A_2, B and C are also considered to be such biphenyl type dimers (biflavanols) (Fig. 3) as suggested by Roberts et al.[2] Meanwhile these catechin dimers were isolated in crystals by Vuataz et al.[5], and later the proposed structures were ascertained by Ferretti et al.[6] on the basis of nmr spectral evidence.

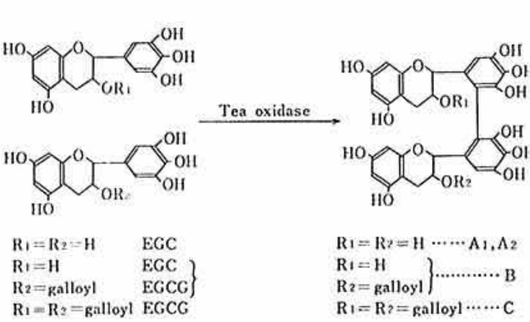

Fig. 3. Dimerization of pyrogallol-type catechins

Formation of benzotropolone pigments

The biflavanols are almost colorless substances, and details of their further oxidative changes are obscure. However, when EGC was incubated with tea oxidase together with EC, namely in the oxidation of a mixture consisted of two catechins having different hydroxylation pattern, it was found that a remarkable red color developed, and the paper chromatogram showed an orange spot P. The corresponding pigment (P) was also found to be formed by the oxidation of a mixture of EGC and EC with an oxidizing reagent consisted of potassium ferricyanide and sodium hydrogen carbonate, and it was isolated as reddish orange needles, for which structure P(R_1=R_2=H) presented in Fig. 4, was proposed from a consideration of its chemical properties[7,8]. The same pigment was also isolated from tea infusion after incubation with *Aspergillus niger* to convert coexisting ester type pigments to free state and the identity of the isolated pigment (P_1, theaflavin) with P was confirmed[9]. The fact indicates that the reddish orange pigment (P_1) in black tea is formed from both catechins, EGC and EC, by the action of tea oxidase during the fermentation process. In this reaction the *o*-dihydroxyphenyl group of EC is considered to combine with the *vic*-trihydroxyphenyl group of EGC, forming a novel benzotropolone nucleus as illustrated in Fig. 4. As substantial proof for the formation of such benzotropolone nucleus by the oxidative coupling of two types of hydroxyphenyl groups, the preparation of several related benzotropolonic pigments, i.e. categallin (Q) from catechol and EGC[10] (Fig. 5), pyrogallin(R) from pyrogallol and EGC[10] (Fig. 5), erycitrin (erycetin-rhamnoside) from catechol and myricitrin[11] (Fig. 6), and pigment S from (+)-catechin and gallic acid (Fig. 7), could be accomplished, and the following oxidative degradation with alkaline hydrogen peroxide afforded additional information supporting the proposed benzotropolonic structures of pigments P, Q, R, S and erycetin[12]. It has been known that purpurogallin-4-carboxylic acid having a 2′, 3′, 4′-trihydroxybenzotropolone nucleus affords α, β′-dicarboxy-β-carboxymethyl tropolone on oxidizing with hydrogen peroxide in alkaline solution. Formation of the same product from pyrogallin was also recognized by chromatographic analysis. On the other hand, it was found that the pigments having 3′, 4′-dihydroxybenzotropolone nucleus, such as theaflavin, erycetin, 3′, 4′-dihydroxybenzotropolone-4-carboxylic acid and pigment S, gave 6-hydroxybenzene-1, 2, 4-tricarboxylic acid. The fact proves that the product originates in the tropolone ring of the 3′ 4′-dihydroxybenzotropolone nucleus, being accompanied with the transformation of the seven-membered ring to the six-membered benzene ring during the oxidative degradation.

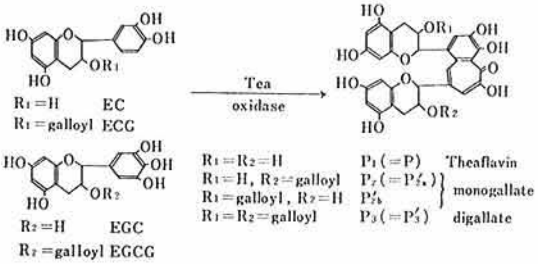

Fig. 4. Formation of theaflavin and its gallates

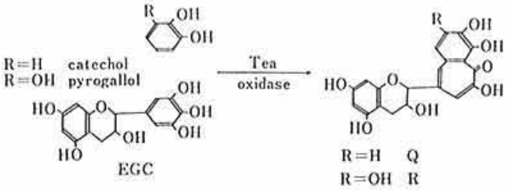

Fig. 5. Formation of categallin (Q) and pyrogallin (R)

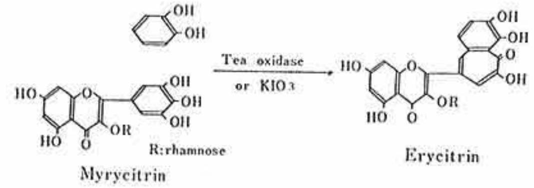

Fig. 6. Formation of erycitrin

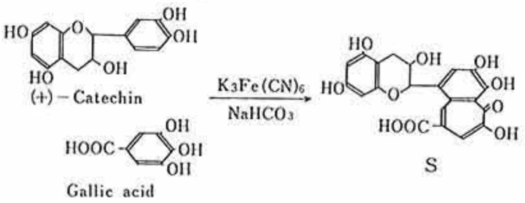

Fig. 7. Formation of pigment S

More conclusive evidence for the correctness of the structures proposed for theaflavin, categallin, pyrogallin and erycetin were obtained by nmr and mass spectrometry[13-15].

Crystalline theaflavin gallates of black tea

Chromatographic analysis of tea infusion indicates the presence of two remarkable orange spot P_1(=P) and P_2, the latter being much larger. Roberts[16] also indicated the presence of theaflavin gallate in black tea together with free theaflavin. Vuataz et al.[5] fractionated the polyphenolic substances of black tea by cellulose column chromatography and found other pigment Y_{II} to be contained beside the above two pigments. In order to understand the real nature of the pigment gallates, attempts were made to isolate them in crystalline form from black tea and elucidation of their structures was carried out[17]. By means of solvent extraction and column chromatography colored substances P_2 and P_3 were isolated in reddish orange needles from black tea manufactured from the leaves of variety "Benihomare". These pigments were hydrolyzed by tannase quantitatively to free theaflavin and gallic acid. The spectral evidence and synthetic approach by means of the oxidation of catechin gallates confirmed that P_2 was theaflavin monogallate formed by the coupled oxidation of EC and EGCG, while P_3 was theaflavin digallate formed by the coupled oxidation of ECG and EGCG. The occurrence of another theaflavin monogallate P_{2b}', which is synthesized from ECG and EGC, a positional isomer of P_{2a}', in black tea seems to be probable, but is still inconclusive. Recently minor constituents, isotheaflavin, epitheaflavic acid and its gallate, were added to the group of tea benzotropolonic pigments[18].

In practical use of tea it is noticeable that the reddish orange pigments theaflavins having benzotropolone nucleus contribute a great deal to the development of bright reddish color of aqueous tea-infusions.

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