The Biochemistry of Tea Fermentation: Oxidative Degallation and Epimerization of the Tea Flavanol Gallates

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Degallation and epimerization of the tea flavanols (−)-epigallocatechin gallate and (−)-epicatechin gallate were shown to take place when these compounds were incubated with tea catechol oxidase. Both flavanols were epimerized at the C-2 position under anaerobic and aerobic conditions in the presence of the active enzyme. Under oxidative conditions, about 2% of the oxidized (−)-epigallocatechin gallate molecules undergo degallation with the formation of free gallic acid and tricetinidin, whereas about 10% of the oxidized (−)-epicatechin gallate molecules are degallated with the formation of free gallic acid and a mixture of C-2 and C-3 epimeric flavanols. These results suggest that the specific catechol oxidase mediated oxidation of the ortho-diphenolic group on the B-ring in tea flavanols induces electron displacements over the flavanol molecules, producing some oxidative changes remote from the B-ring.

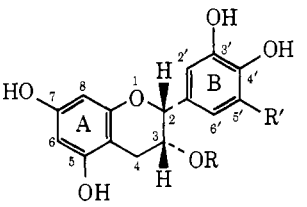
Tea fermentation (Roberts, 1962; Sanderson, 1972) is an essential part of the process by which fresh green tea leaf is converted to the black tea of commerce. Central to this so-called fermentation is the oxidation of the tea flavanols (I–IV) which is catalyzed by an endogenous catechol oxidase. The tea flavanols comprise 20 to 30% of the dry weight of fresh tea leaf tissues (Vuataz *et al.*, 1959) and these compounds are almost completely oxidized during the tea fermentation process (Vuataz and Brandenberger, 1961). Apart from their interesting chemistry, the oxidation products of the tea flavanols are of considerable practical importance because they are responsible for the color of black tea (Sanderson, 1972) and they are involved in determining the taste (Millin *et al.*, 1969) as well as the solubility (Takino, 1971) of black tea infusions.

Previous investigations into the chemistry of the flavanol oxidation products formed during tea fermentation have established the reactions leading to the formation of theaflavins, bisflavanols A (XI), B (XII), and C (XIII), epitheaflavic acid (XIV), and 3-galloyl epitheaflavic acid (XV). This work has recently been reviewed (Sanderson, 1972; Sanderson *et al.*, 1972). There are still, however, several unidentified compounds which are known to be formed during tea fermentation (Nakagawa and Torii, 1965; Roberts, 1962; Sanderson *et al.*, 1972).

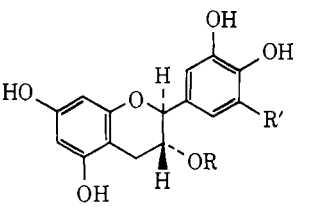
It was the object of this investigation to improve our understanding of the nature of the oxidation products of the gallated flavanols, namely, (−)-epigallocatechin gallate (IV) and (−)-epicatechin gallate (II), which account for about 60% of all the flavanols in fresh tea leaves. In a recent investigation (Coggon *et al.*, 1973), it was noticed that some degallation and epimerization of the gallated tea flavanols took place when these compounds underwent oxidation mediated by tea catechol oxidase. Some epimerization of tea flavanols (Dzhemukhadze *et al.*, 1964) and formation of free gallic acid (Roberts and Wood, 1951; Takino, 1970) during tea fermentation has been reported. These reactions have now been studied in more detail and, as shown below, the results of these studies have contributed to the further characterization of the products of tea fermentation.

EXPERIMENTAL SECTION

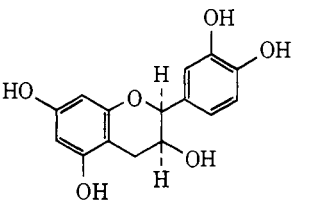
Materials. Fresh tea flush, *i.e.*, the tea shoot tips to just below the second leaf, was air-freighted to us from Lipton's Experimental Tea Garden near Charleston, S. C., and was stored at −40° until required. (−)-Epigallocatechin (III), II, and IV were prepared from fresh frozen green tea leaf as described previously (Co and Sanderson, 1970). (−)-Epicatechin (I) and (+)-catechin (X) were purchased from Pierce Chemical Co., Rockford, Ill. (−)-Gallocatechin gallate (VIII) was a gift from C. K. Wilkins, Unilever Research Laboratory, Vlaardingen, The Nether-



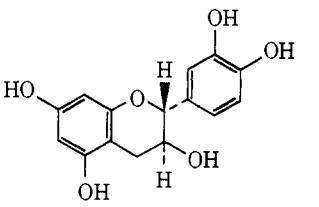
I, R = R′ = H
II, R = galloyl; R′ = H
III, R = H; R′ = OH
IV, R = galloyl; R′ = OH



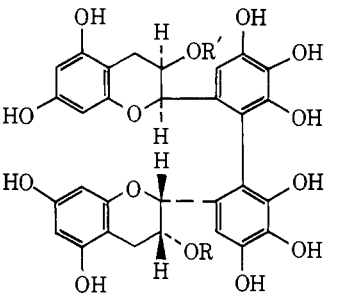
V, R = R′ = H
VI, R = galloyl; R′ = H
VII, R = H; R′ = OH
VIII, R = galloyl; R′ = OH



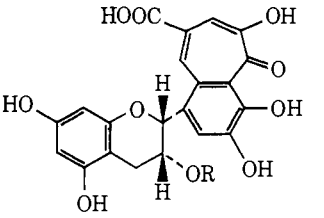
IX



X



XI, R = R′ = galloyl
XII, R = galloyl; R′ = H
XIII, R = R′ = H



XIV, R = H
XV, R = galloyl

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Table I. Products Obtained from (−)-Epigallocatechin Gallate (IV) Reacted with Purified Tea Catechol Oxidase under Aerobic and Anaerobic Conditions (See Figure 1 for Paper Chromatographic Identification of Phenolics)

Reaction time, min	Phenolic compounds present in reaction mixture[a]					
	(−)-Epigallocatechin gallate (IV)	(−)-Gallocatechin gallate (VIII)	Gallic acid	Tricetinidin (XVIII)	Bisflavanols (XI, A′, A″)	Thearubigins
Experiment A: reaction carried out under aerobic conditions						
0	++++	—	—	—	—	—
15	+++	++	tr	tr	—	—
30	++	+	+	+	tr	tr
60	+	—	++	++	++	++
60 (with heat-inactivated enzymes)	++++	—	—	—	—	—
Experiment B: reaction carried out under anaerobic conditions						
0	++++	—	—	—	—	—
15	+++	+	—	—	—	—
30	++	++	—	—	—	—
60	++	++	—	—	—	—
60 (with heat-inactivated enzymes)	++++	—	—	—	—	—

[a] Quantifications indicated are estimates of spot intensities on paper chromatograms. Key to abbreviations used: ++++ = very dark spot; +++ = dark spot; ++ = medium spot; + = light spot; tr = trace spot; — = no spot.

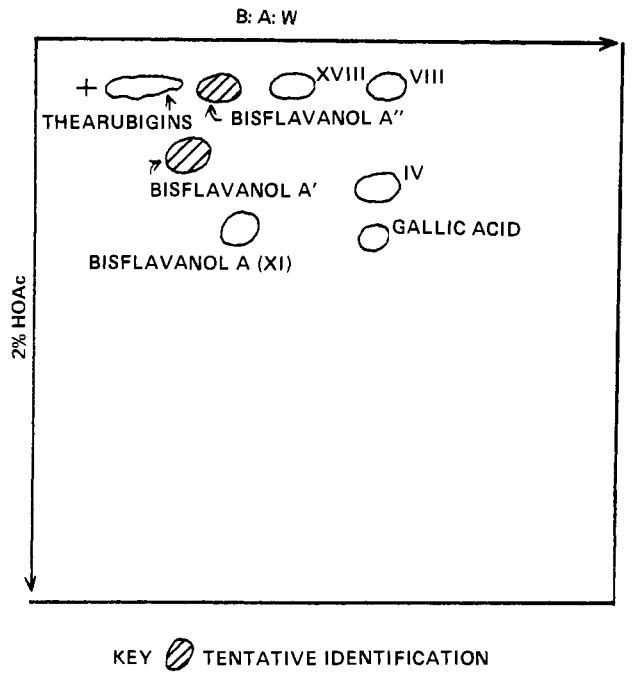


Figure 1. Composite chromatogram of oxidation products of (−)-epigallocatechin gallate (IV).

lands. Crude soluble tea (CST) enzymes and purified tea catechol oxidase were prepared from fresh frozen green tea leaf and assayed for catechol oxidase, as described by Coggon *et al.* (1973).

Model Tea Fermentation Systems. The model systems consisted of 2.5 ml of 8.7 m*M* tea flavanol in 0.1 *M* phosphate–citrate buffer, pH 5.7, plus 1.0 ml of tea enzyme preparation adjusted to contain 1 unit of catechol oxidase activity. Incubations were carried out at 30° either in open shaking test tubes (aerobic incubation) or in evacuated Thunberg tubes (anaerobic incubation).

The enzymic oxidation of IV was repeated in the presence of ascorbate, which acts as a reducing agent (El-Bayoumi and Frieden, 1957). The reaction mixture consisted

Table II. Effect of Preventing the Formation of Oxidation Products upon the Oxidative Degallation of (−)-Epigallocatechin Gallate (IV) (See Results Section for an Explanation of the Role of Ascorbic Acid)

Reaction time, min	Oxygen uptake, μmoles	Ascorbic acid present, μmoles	Phenolic compounds present[a]		
			(−)-Epigallocatechin gallate (IV)	Gallic acid	Oxidation products
0	0	13.4	++++	—	—
5	2.2	9.0	++++	—	—
10	4.5	4.4	++++	—	—
15	6.6	0.2	++++	tr	tr
20	8.8	0.0	+++	+	+

[a] See Table I footnote.

of 2.5 ml of 2.5 m*M* EGCG, 2.5 ml of 5.3 m*M* ascorbate in 0.05 *M* citrate–phosphate buffer, pH 5.7, and 1.0 ml of purified tea catechol oxidase containing 1 unit of enzyme activity. The oxidation was carried out in a Gilson differential respirometer. Ascorbic acid oxidation was estimated from the amount of oxygen consumed assuming the following reactions.

$$\tfrac{1}{2}\,O_2 + IV \rightarrow XVI + H_2O$$

$$XVI + \text{ascorbic acid} \rightarrow \text{dehydroascorbic acid} + IV$$

Reactions were terminated by immersing reaction flasks in boiling water for 5 min to inactivate the enzymes. Reaction products were identified by paper chromatography (Coggon *et al.*, 1973), and quantified by gas chromatography.

Gas–Liquid Chromatography for Quantitative Determinations. Flavanols and gallic acid were determined by a modification of the procedure described by Collier and Mallows (1971) using a Barber-Coleman Model 5000 gas chromatograph equipped with dual columns and flame ionization detectors. Samples were derivatized with *N,O*-bis(trimethylsilyl)acetamide in pyridine and chromatographed on a 10 ft × ⅛ in. stainless steel column packed with 3% OV-1 on 60–80 mesh Gas Chrome Q conditioned at 330°. The operating parameters were: injection temper-

Table III. Quantitative Data for the Oxidative Degallation of (−)-Epigallocatechin Gallate (IV) and (−)-Epicatechin Gallate (II)

Length of reaction, min	Phenolic compounds found in reaction mixture (μmoles)					
	(−)-Epigallocatechin gallate (IV)	(−)-Epicatechin gallate (II)	(−)-Epicatechin (I) + (+)-catechin (X)	Tricetinidin (XVIII)	Gallic acid	Others
	Experiment A: EGCG model system with IV as substrate					
0	220	—	—	0	0	None
60	96	—	—	2.6	2.8	Several (Figure 1)
	Experiment B: ECG model system with II as substrate					
0	—	226	0	—	0	None
60	—	95	10	—	13	Several (Figure 2)
	Experiment C: whole tea flush samples (1.0 g dry weight basis)[a]					
0 (dried green)	284 (13)[b]	101 (4.5)	68 (2.0)	0	6 (0.1)	Several[c]
90 (black tea)	tr	tr	tr	2.4 (0.07)	29 (0.5)	Several[c]

[a] Values for I, II, IV, and X taken from Pierce *et al.* (1969). [b] Numbers in parentheses are percentages on 1.0 g of total tissue dry weight basis. [c] See figures in Sanderson (1972).

ature, 310°; column, 240–305° at 10°/min (180° isothermal for gallic acid); detector temperature, 330°; and helium flow rate, 36–40 ml/min. Under these conditions the following retention times were obtained: IV, 27 min; II, 24 min; I, 16 min; and gallic acid, 4 min. Quantitation was accomplished by comparison with known amounts of authentic compounds using appropriate internal standards.

Isolation, Identification, and Quantitation of Tricetinidin (XVIII). A model system oxidation of 200 mg of IV with CST enzymes was terminated after 60 min by heat denaturation, and the solution was divided into two portions. One was analyzed by gas chromatography for IV and gallic acid, while the second was extracted with 6 × 50 ml of ethyl acetate. The combined organic layers from the latter portion were concentrated to 200 μl. XVIII was isolated by paper chromatography using Whatman 3 MM paper. The pink spot at R_f 0.50 (1st dimension, B:A:W):0.00 (2nd dimension, 2% acetic acid) was eluted with ethanol (Eshadt and Mirelman, 1972). The eluate was dried to yield 1.0 mg of crude XVIII. The sample was methylated by dimethyl sulfate–potassium carbonate in refluxing acetone. Under these basic pH conditions, XVIII ring-opens (Jurd, 1963) to give the chalcone XIX. After filtering, evaporating, and treatment with ammonia, the yellow solid was further purified by tlc using silica gel and benzene–methanol (3:1). After Soxhlet extraction and evaporation, the yellow material (0.6 mg) was compared with hexamethyl XIX synthesized by the method of Jurd (1963). The two samples were shown to be identical by tlc, ir, nmr, and elemental analysis. XIX was quantified after methylation by its uv absorption in chloroform at $\lambda = 365$ nm ($\epsilon = 4.6 \times 10^4$).

RESULTS

Products Obtained from (−)-Epigallocatechin Gallate (IV) Reacted with Tea Catechol Oxidase. IV was incubated with purified tea catechol oxidase in a model tea fermentation system described in the Experimental Section, and the products formed were studied by paper chromatography. The results (Experiment A, Table I) showed that an array of products was formed under aerobic conditions. These products were chromatographically located, as shown in Figure 1, and the results indicated that the following relationship existed between the products: (−)-gallocatechin gallate (VIII) was formed by epimerization of IV at the C-2 position. This epimerization also occurred under anaerobic conditions (Experiment B, Table I), in which case it was the only noticeable reaction which took place. In both cases, the epimerization was dependent on the presence of active tea catechol oxidase.

It is noteworthy that VIII cochromatographs with theaflavin gallate in the paper chromatographic system (Roberts *et al.*, 1957) used in this investigation. This fact may explain why, in their early work, Roberts and Myers (1959) contended that theaflavins were formed from IV alone. It should be recognized, however, that Roberts (1962) was, after all, the first to establish the correct precursors of theaflavins.

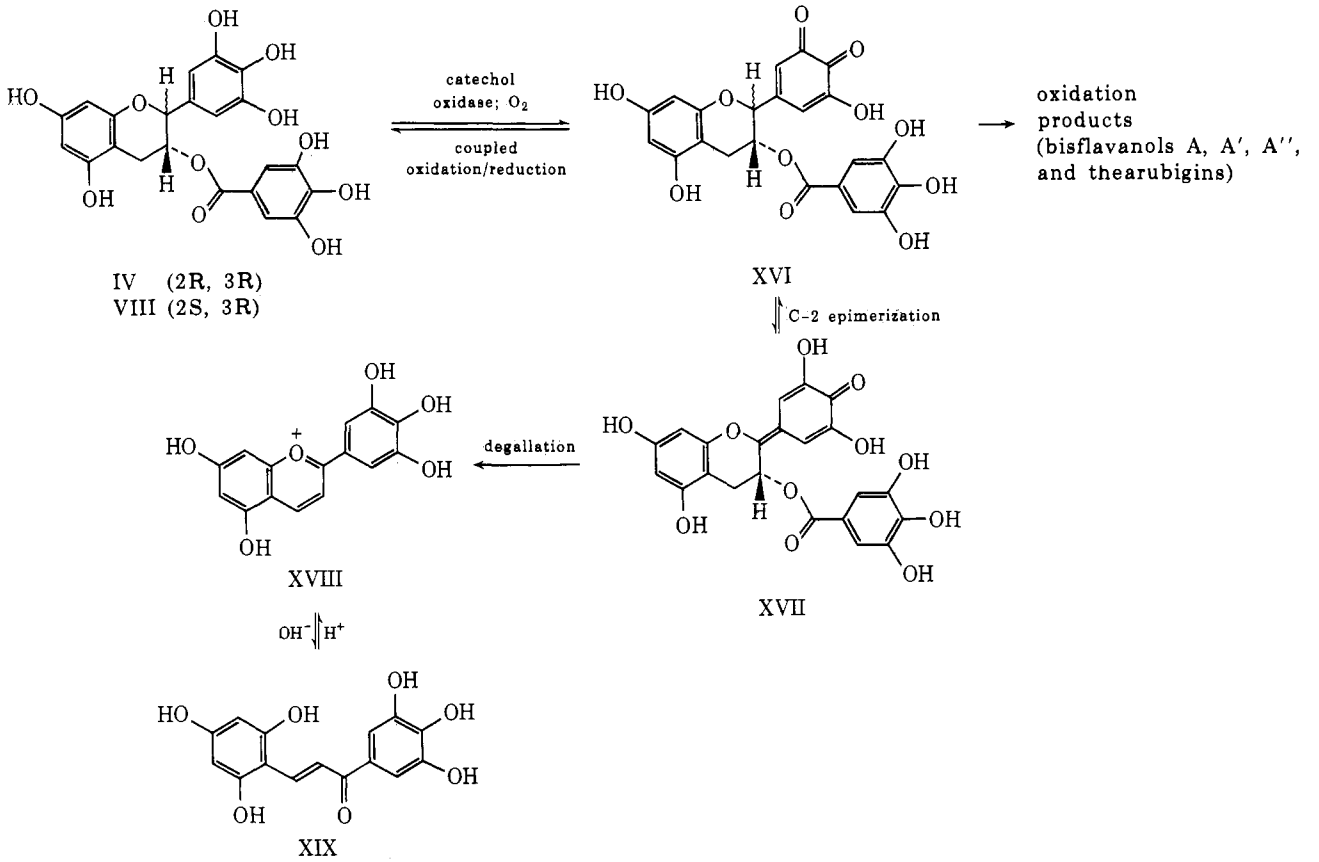
Gallic acid is one of the products formed when IV is oxidized by tea catechol oxidase (Experiment A, Table I). The dependence of this degallation on the oxidation of IV was established in two ways. First, no gallic acid was formed under anaerobic conditions (Experiment B, Table I); this rules out the possibility of an esterase (tannase) being present as a contaminant in the purified tea catechol oxidase preparation. Second, an experiment was carried out in which a limited amount of ascorbic acid was added to the reaction mixtures to prevent the formation of IV oxidation products from IV during the initial portion of the reaction period (see Experimental Section); the results showed (Table II) that no gallic acid was formed until the ascorbic acid was used up and oxidation products of IV began to form.

Tricetinidin (XVIII) is formed whenever IV is oxidatively degallated. This was confirmed chromatographically by the consistent appearance of XVIII and gallic acid together whenever IV underwent oxidation, and by a quantitative determination of XVIII and gallic acid which showed (Experiment A, Table III) that essentially equimolar quantities of these two products are formed on oxidation of IV. Further, these results show that only about 2% of the oxidized IV molecules undergo oxidative degallation. Finally, (−)-epigallocatechin (III), a product expected from the simple deesterification of IV, was not formed (Table I) under any of the conditions studied in this investigation. Our results confirm and explain the report by Roberts and Williams (1958) that Substance P (which they found in trace quantities in black tea) was the flavylium salt XVIII and that Substance P was probably formed from IV.

Bisflavanol A (XI) which is formed by the oxidative condensation of IV (Roberts, 1962; Sanderson, 1972) was a major oxidation product (Table I), but the presence of VIII leads us to believe that two faint unidentified spots on our chromatograms (Figure 1) are due to the epimeric bisflavanols A′ and A″ formed from IV plus VIII and 2 VIII, respectively.

Finally, some thearubigins were formed (Table I; Figure 1) on oxidation of IV. These brown-colored substances are as yet only poorly characterized as polymeric proanthocyanidins (Brown *et al.*, 1969a,b) and these results show again (Nakagawa and Torii, 1965; Sanderson *et al.*, 1972)

Scheme I
Oxidation Pathway for Gallocatechins



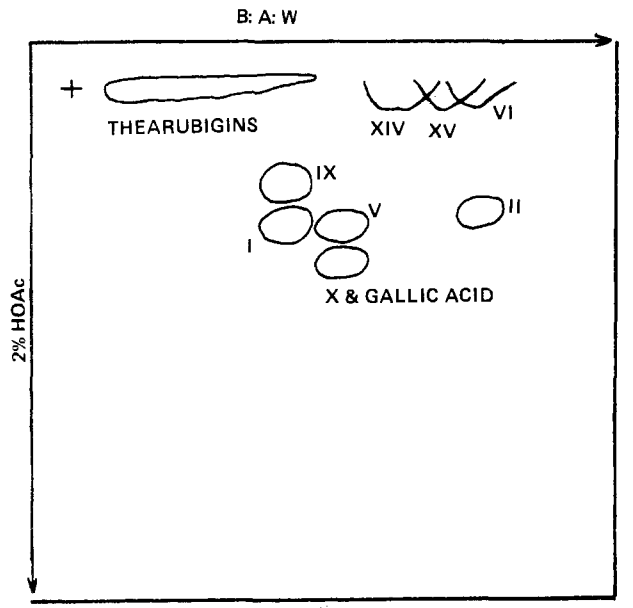


Figure 2. Composite chromatogram of oxidation products of (−)-epicatechin gallate (II).

that the thearubigins are formed as products of the oxidation of tea flavanols (I–IV) singly or in any combination.

The above results obtained for the oxidation of IV are summarized in Scheme I.

Products Obtained from (−)-Epicatechin Gallate (II) Reacted with Tea Catechol Oxidase. The oxidation of II by tea catechol oxidase was studied in the same way as described above for IV. The results (Table IV; Figure 2) may be summarized as follows. First, epimerization at the C-2 position of II was shown to be catalyzed by tea catechol oxidase, as evidenced by the formation of (−)-catechin gallate (VI). As with IV (Table I), this epimerization takes place under both aerobic and anaerobic conditions in the presence of active tea catechol oxidase.

Gallic acid is formed as a result of the oxidation of II (Experiment A, Table IV), and the possibility that this degallation is due to an esterase (tannase) activity is again ruled out by the finding that degallation does not occur under anaerobic conditions (Experiment B, Table IV). However, the degallation of II appears to lead to the formation of the corresponding degallated flavanol I and its C-3 epimer (+)-catechin (X) rather than the analog of tricetinidin (XVIII), as might be expected from the results obtained with IV. Also, quantitative measurements (Experiment B, Table III) showed that approximately equimolar quantities of gallic acid and I plus X were formed during the oxidation of II, providing further evidence for these compounds being the products of the oxidative degallation of II. These quantitative data (Table III) also show that about 10% of the oxidized II molecules undergo oxidative degallation.

In separate model system experiments, I and X were epimerized by incubating these compounds under anaerobic conditions with CST enzymes to form mixtures of I plus V and IX plus X, respectively. These mixtures were oxidized and analyzed at 5-min intervals. V and IX were rapidly oxidized and disappeared completely before I and X. These results suggest that V and IX will not be found in appreciable quantities in oxidized reaction mixtures of II (Table IV) because their reactivity prevents their accumulation.

Table IV. Products Obtained from (−)-Epicatechin Gallate (II) Reaction with Crude Tea Catechol Oxidase under Aerobic and Anaerobic Conditions (see Figure 2 for Paper Chromatographic Identification of Phenolics)

Reaction time, min	Phenolic compounds present in reaction mixture[a]					
	(−)-Epicatechin gallate (II)	(−)-Catechin gallate (VI)	Gallic acid	(−)-Epicatechin (I)	(+)-Catechin (X)	Oxidation products
	Experiment A: reaction carried out under aerobic conditions					
0	++++	—	—	—	—	—
30	++	+	+	tr	tr	+
30 (with heat-inactivated enzymes)	++++	—	—	—	—	—
	Experiment B: reaction carried out under anaerobic conditions					
0	++++	—	—	—	—	—
30	+++	+	—	—	—	—
30 (with heat-inactivated enzymes)	++++	—	—	—	—	—

[a] See Table I footnote.

Some 3-galloyl epitheaflavic acid (XV) is formed in this system (Table IV) as would be expected (Berkowitz *et al.*, 1971) in an oxidizing system which contains II and gallic acid. Of course, as discussed above, the gallic acid required is formed by the oxidative degallation of II. It is probable that epitheaflavic acid (XIV) is also present among the reaction products because of the presence of small amounts of I in these reaction mixtures, but this was not confirmed inasmuch as XIV and XV are only poorly resolved in the chromatographic system used (Figure 2). Again, some thearubigins are formed from the oxidation of II (Table IV).

The above results obtained for the oxidation of II are summarized in Scheme II.

Oxidative Degallation during Tea Fermentation. The fresh green tea flush of the type used in this investigation has been found to contain about 13% IV and 4.5% II on a dry weight basis (Figure 3 in Pierce *et al.*, 1969). When this tea flush is converted to black tea by conventional manufacturing procedures (Sanderson, 1972), most of the simple tea catechins are converted to various oxidation products. Calculations based on the model system studies described above (Table III) show that this conversion of fresh green tea leaf to black tea may be expected to be accompanied by an increase in the free gallic acid level of about 0.3% by weight due to oxidative degallation. In fact, analyses of samples of dried green tea flush and black tea made from this flush show (Experiment C, Table III) that this conversion is accompanied by an increase in gallic acid amounting to about 0.4%. The extra gallic acid (*i.e.*, ~0.1%) may well be accounted for by the low level of endogenous tannase enzyme found in this tea leaf (Coggon *et al.*, 1973). Indeed, when IV and II were treated separately with CST enzymes under anaerobic conditions for 60 min, gallic acid was released to the extent of 0.2 and 0.8%, respectively, which is equivalent to about 0.06% gallic acid on a tea flush dry weight basis. The 0.07% tricetinidin found in this set of samples was only about half that expected from the amount of IV estimated to have been oxidized.

DISCUSSION

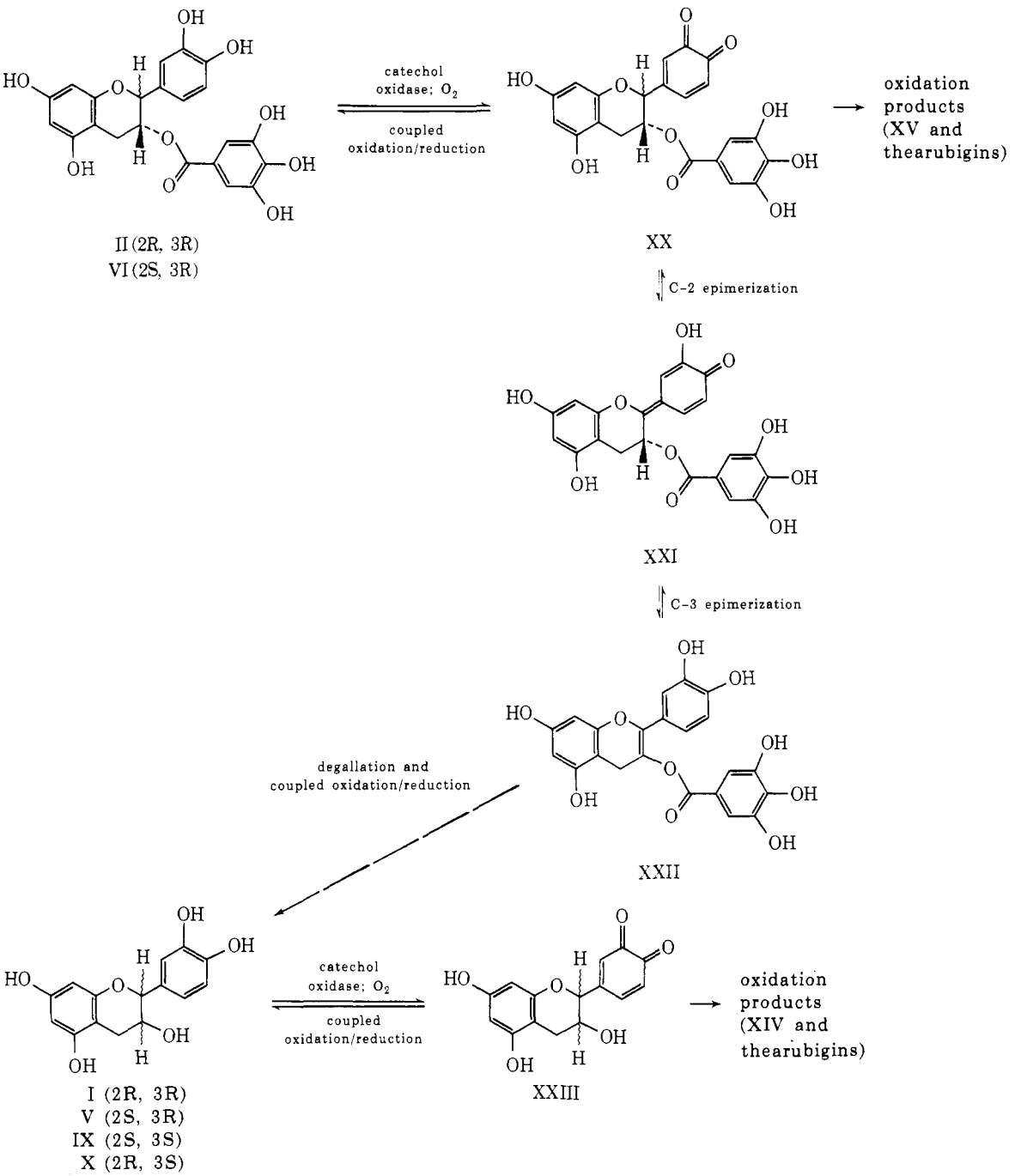
The results of this investigation (summarized in Schemes I and II) establish that some degallation of the gallated tea flavanols (II, IV) results from the oxidation of these compounds mediated by tea catechol oxidase. Surprisingly, it was found that the oxidation products were not analogous. The identification of tricetinidin (XVIII) as the coproduct with gallic acid of the oxidative degallation of (−)-epigallocatechin gallate (IV) appears to require a rearrangement of the ortho-quinone (XVI) formed when IV undergoes catechol oxidase mediated oxidation. A mechanism to explain these results is proposed in Scheme I. This mechanism suggests that epimerization takes place under oxidative conditions in competition with other reactions, such as the formation of bisflavanols and thearubigins. The epimerization of IV which takes place under these conditions should lead to the formation of (−)-gallocatechin gallate (VIII) and thereby to epimeric oxidation products such as bisflavanols A′ and A″ discussed above, as well as various theaflavins. Although many theaflavins have been characterized (Collier *et al.*, 1973), the theaflavins containing VIII have not been reported. The fact that all of the epimers of the well-characterized tea flavanol oxidation products have not yet been reported may be due to the relatively small amounts of these compounds which would be expected to form during tea fermentation.

A further novel configuration of the bisflavanols (XI–XIII) isolated from black tea (Ferretti *et al.*, 1968) may be proposed from a consideration of the hindered rotation of the biphenyl groups. Hindered rotation about the sp^2–sp^3 hybridized C–C bond between 4,6- and 4,8-linked bisflavanoid molecules has been reported by du Preez *et al.* (1971), but hindered rotation of the biphenyl of tea bisflavanols has not yet been reported. Two isomers should be present for each bisflavanol of fixed configuration at C-2 and C-3. These isomers may be separable by paper chromatography. Indeed, it is likely that the pair of bisflavanol products A_1 and A_2 formed from III reported by Takino and Imagawa (1963, 1964) are examples of this type of isomerism.

The oxidative degallation of (−)-epicatechin gallate (II) appears to be more difficult to explain because the products of this reaction are apparently equimolar quantities of gallic acid and the degallated flavanols I and X. Of course, these compounds can be further oxidized in this system to epitheaflavic acid (XIV) and thearubigins. These results appear to require that oxidized II molecules undergo deesterification and reduction in a concerted and linked reaction. The mechanism shown in Scheme II is proposed to account for these results.

The mechanisms proposed for the oxidation of II and IV by tea catechol oxidase appear to be consistent with the information available in the literature. The work of Pelter *et al.* (1971) on flavanols and Jurd (1972a,b) on flavylium salts certainly suggests that extensive delocalization of charge does occur in molecules of this type and that this delocalization plays an important role in determining the reactions which follow.

Scheme II
Oxidation Pathway for Catechol Catechins



The catechol oxidase mediated epimerizations that were found to take place also appear to be accounted for in the reaction mechanisms proposed in Schemes I and II. First, epimerization at C-2 of both II and IV occurs in the presence of active tea catechol oxidase under both aerobic and anaerobic conditions. Anaerobic epimerization is not fully understood, but this may result from a transitory and reversible transfer of electrons to the enzyme when the enzyme and substrate are in the form of an activated complex. This transfer of electrons must be sufficiently complete to allow the formation of oxidation forms equivalent to XVII (Scheme I) and XXI (Scheme II). This allows epimerization at C-2. However, the absence of oxygen (the natural electron acceptor) prevents dissociation of the enzyme-substrate complex unless the flavanols are in their reduced form. Under these conditions, an equilibrium is established among the enzyme, the two C-2 epimers of the flavanol involved, and the activated flavanol-enzyme complex.

Epimerization at the C-3 position only occurs with II when oxidative degallation is taking place. Even then, only two, namely I and X, of the four possible epimers are formed in appreciable quantities. The absence of V and IX may be explained by their rapid oxidation by tea catechol oxidase. Of course, the absence of flavanol epimerization at the C-3 position in the oxidative degallation of IV is explained by the fact that XVIII with no chirality is, together with gallic acid, the product of this reaction.

Nakagawa (1967) has shown that the tea flavanols (I–IV, X) are epimerized at the C-2 position, and that the

gallated tea flavanols (II, IV) are also degallated during a heating step in the manufacture of Hoji-cha green tea. Chromatographic data given by Nakagawa (1967) for Unknowns 3 and 4 formed from II and IV, respectively, suggest that Unknown 3 ≡ (−)-catechin gallate (VI) and that Unknown 4 ≡ (−)-gallocatechin gallate (VIII). If this is so, the epimerization and degallation reactions undergone by tea flavanols during green tea manufacture are identical to those which take place during black tea manufacture. However, these green tea reactions are thermally induced, since the tea leaf catechol oxidase is inactivated during an initial step of the green tea manufacturing process. Evidently, the acidic conditions (~pH 4.8) existing in tea leaves, the metals present, and the concentration of the reactants are sufficient to catalyze these changes at the temperatures employed; *i.e.*, 100 to 150°.

It is noteworthy that potassium ferricyanide and tea catechol oxidase catalyze the formation of the same type of oxidation products (Bryce *et al.*, 1970; Coxon *et al.*, 1972a,b; Ferretti *et al.*, 1968; Takino *et al.*, 1964). Collier *et al.* (1973) found that both epitheaflavic acid (XIV) and 3-galloyl epitheaflavic acid (XV) were formed during the oxidation of II and gallic acid by potassium ferricyanide, which suggests that this oxidizing agent does catalyze oxidative degallation of tea catechins in the same way as the tea enzymes system. Evidently, potassium ferricyanide and tea catechol oxidase effect the same type of complexing with tea flavanols, thereby directing the same oxidative results. Certainly, our evidence (Coggon *et al.*, 1973; this investigation) suggests that the small amount of esterase activity present in tea leaves is not an important catalyst of the degallation reactions which take place during tea fermentation, as was suggested by Collier *et al.* (1973).

Considering the multiplicity of reactions known to occur in tea flush during its conversion to black tea (Sanderson, 1972), the agreement found between the amount of oxidative degallation expected based on the model system studies and that found to take place in actual practice (Table III) appears to be surprisingly good. These results suggest that the degallation reactions (Roberts and Wood, 1951; Takino, 1970) and the interrelated epimerizations (Dzhemukhadze *et al.*, 1964) which take place during the black tea manufacturing process are accounted for by the reactions studied in this investigation.

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