

TABLE I
 Minikits by rice varieties

Variety	No. States	Farmers
IET 1991	.. 17	20,360
IET 1039	.. 12	9,960
IR 579-48	.. 4	6,050
Galmidge Resistant Varieties..	10	4,650
IR 20	.. 4	5,600
Vijaya	.. 4	4,150
CR 44-35	.. 6	3,810
14466	.. 1	3,300
New Sabarmati	.. 4	2,950
Basmati Dwarfs	.. 5	2,475
Bala	.. 2	2,000
Pankaj	.. 2	1,700
Tungro Virus Resistant Varieties 5	500
Cauvery	.. 1	500
CR 36-148	.. 1	500
Malinja	.. 1	500
CR 44-1	.. 1	310

variety or technique which has successfully passed through so many sieves becomes a success in the field. This does not mean that a new variety may not succumb suddenly to a new disease or a new strain of an already prevalent pathogen, *e.g.*, this is what happened with the famous miracle rice varieties in the Philippines when they were badly damaged by the Tungro virus outbreak during 1971. This is only a warning that there is no room for complacency and no time to relax as far as agricultural research, education and extension efforts are concerned.

Agriculture is becoming an increasingly sophisticated occupation. With the growing awareness of the ecological dangers arising from the inappropriate and unscientific use of agricultural inputs like pesticides, fertilizer and water, steps are under way to develop techniques like integrated pest control, watershed management, etc., which will ensure that productivity is continuously

enhanced without any danger to the long-term production potential of the soil. We witness today two major kinds of agricultural growth in the world. In one system, like that prevailing in North America and Europe, more and more land is being cultivated by less and less number of people and farming is therefore a labour-displacing occupation. This has been achieved through extensive mechanisation and highly organised management. In contrast, in our country, agriculture has not only to be a source of food to the people but it has also to be for many years to come the most important instrument of economic growth and productive employment. To achieve agrarian prosperity, an increase in the *per capita* productivity is essential.

Modern agriculture rests upon four major revolutions: (i) agronomic (including genetic), (ii) chemical, (iii) engineering and (iv) management. It is only when all these four revolutions are synchronized in a perfectly coordinated manner that agriculture becomes highly efficient and competitive. While we may be justifiably happy at the progress that has been achieved during the last 25 years, the country needs even more significant results in the years to come. The investment on agricultural research and education made in the last few years will start giving a "pay off" only in the next few years. A dynamic production programme can be sustained only by a dynamic research and training programme. Unless the many complex and urgent problems arising from deficiencies in soil fertility, inadequacies in water management, poor pest control, improper storage and marketing arrangements and problems in animal health, nutrition and genetics are all faced and solved soon, the euphoria caused by the Green Revolution will be short-lived. Jawaharlal Nehru's statement "Agriculture cannot wait" is even more relevant today than when it was made 25 years ago.

RECENT ADVANCES IN THE CHEMISTRY OF TEA

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INTRODUCTION

IT is a significant capacity of human beings that though they may be living in far off places having entirely different climates and environments, they have exhibited somewhat the same needs and have made independent discoveries of materials to satisfy these needs. These materials have either

the same chemical components or closely related ones. As an important example of this type may be mentioned beverages used as stimulants in various parts of the world from fairly ancient times. Tea which had its origin in China, coffee which originated from Arabia, cola-nuts from South Africa, maté or Paraguay tea from South America and cocoa, all provide stimulants which

have either caffeine or its near relative theobromine as active principle. In the case of maté the South Americans have used an ingenious method of brewing it and drinking it out of contact with air. The main objective is stimulation of the central nervous system, creating a feeling of well-being and fitness for greater activity. The beverages also seem to act as diuretics probably arising from increased bloodflow.

The use of caffeine is not always considered favourable for human health. It is habit forming and taken in excess can cause disabilities. Moreover, in the use of tea, taste and flavour are important. Tea tannins, a term loosely employed for a mixture of chemical components, play an important part on taste. It is now known that these are composed of catechins, tropolones and proanthocyanidins which are considerably affected by the processing of tea; they have been estimated in various ways. The aroma is largely dependent on volatile components.

More recent work on the chemistry of tea has utilised modern methods of analysis which are capable of great precision and effective separation. Consequently a large volume of new results have been obtained during the past fifteen years. Compounds belonging to the group of flavonoids and their O- and C-glycosides, some biflavanols, coumarins, gallic and ellagic acids and their derivatives have been obtained. They constitute an impressive list of important components. The presence of saponins and protein complexes have also been noted. Many of these play an important part on the consistency (body and thickness) of tea brew.

There is reason to believe that they all have significant physiological properties. Some of the components have already been studied to some extent. Flavonoids and their glycosides have Vitamin P action. Some biflavonoids regulate blood-flow but detailed study of this aspect of tea has not been adequately made. In the following paragraphs the important chemical components belonging to each of the categories mentioned above are briefly discussed. A few reviews have appeared on the chemistry of tea earlier; this present review lays emphasis on the recent advances in the chemistry of the polyphenolic components of tea.

PART I. MANUFACTURE OF BLACK AND GREEN TEA

The quality of made tea is dependent not only on the soil in which the plant is grown, but also on the methods of manufacture in the factory, and even small errors seriously affect the best

grown tea. The manufacture of black tea is a complex process. After the tender tea leaves (preferably a bud and two leaves) are plucked from the fields and brought to the factory, there are several stages in the processing. They are (i) withering, (ii) rolling and/or CTC machining, (iii) putting in rotorvane, (iv) fermenting and finally (v) drying. The first step withering is intended to take away moisture from the leaf so that it becomes less brittle, will roll well and take on a twist. Withering is generally done by spreading the leaves in troughs which are allowed to remain for some time under controlled conditions of humidity and temperature in a steady but gentle stream of air. The withered leaves are then rolled or distorted in a roller where they undergo a twisting action and the macerated leaves are now-a-days passed through a rotorvane for further distortion. In many factories in recent times the withered tea leaves are passed through CTC machines designed to crush, twist and curl the leaves. By either process the withered tea leaves are mangled and at this stage are known as 'Tea Dhools'. All these operations are designed as preliminary and preparatory to the next step fermentation and this is the core of the whole manufacture. Methods of fermentation are age-old and have not undergone any significant change from the early times. The basic method has been to spread the tea dhools on the floor, in as thin layers as possible and to allow them to ferment in a humid and cooled atmosphere. The process is static. But recently a new method of fermentation using rotating drums has been introduced; this method has been claimed to yield uniformly good grade of tea. The fermented tea dhoool is then passed through a drier where further changes are arrested, the wet tea is dried and made capable of withstanding long storage and transit. On account of the efficient fermentation the black tea differs markedly from that of the original green leaves.

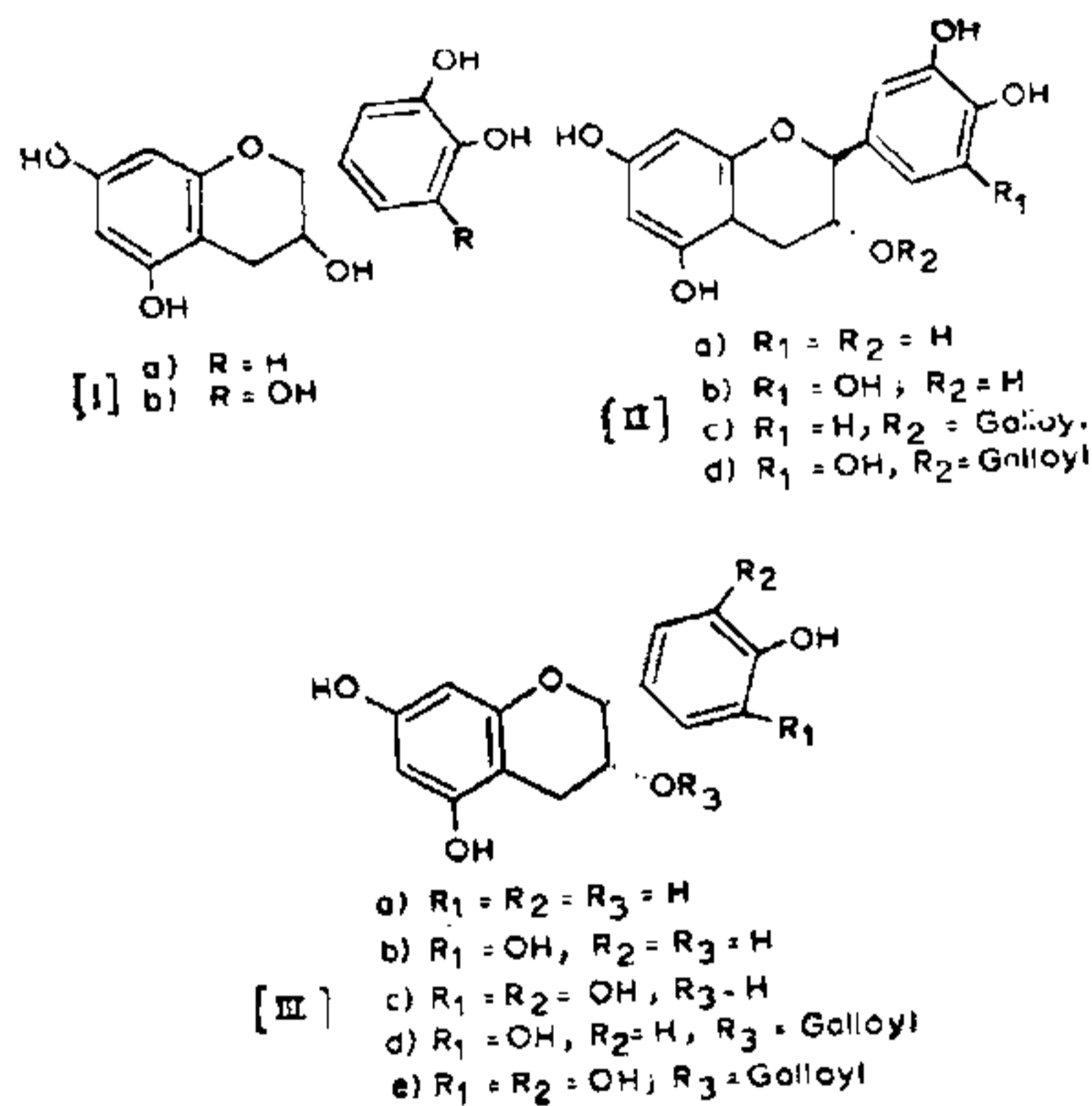
Green tea is made from leaves plucked from the bush in the same manner as the leaves used for black tea. The process is different in that withering and fermentation are completely omitted. The leaves are directly heat-treated, rolled and dried. The heat treatment consists in either pan-firing or steaming to inactivate the enzymes. The heating is completed within a few minutes and the treated leaf is kept bright green without any sign of scorching or reddening. The leaf is then rolled as in the case of black tea and taken immediately to the dryer. The product made by pan-firing is said to be of better quality than that made by

steaming. It will be clear that the conditions employed do not permit any chemical change in the tea leaves and green tea has practically the same composition as the original leaves.

PART II. POLYPHENOLIC COMPONENTS OF TEA

A considerable amount of work has been done on the identification of the polyphenols of tea leaves and made tea. A number of flavanols, flavonols, leucoanthocyanidins and organic acids have been identified in green tea; the main components identified so far are listed below.

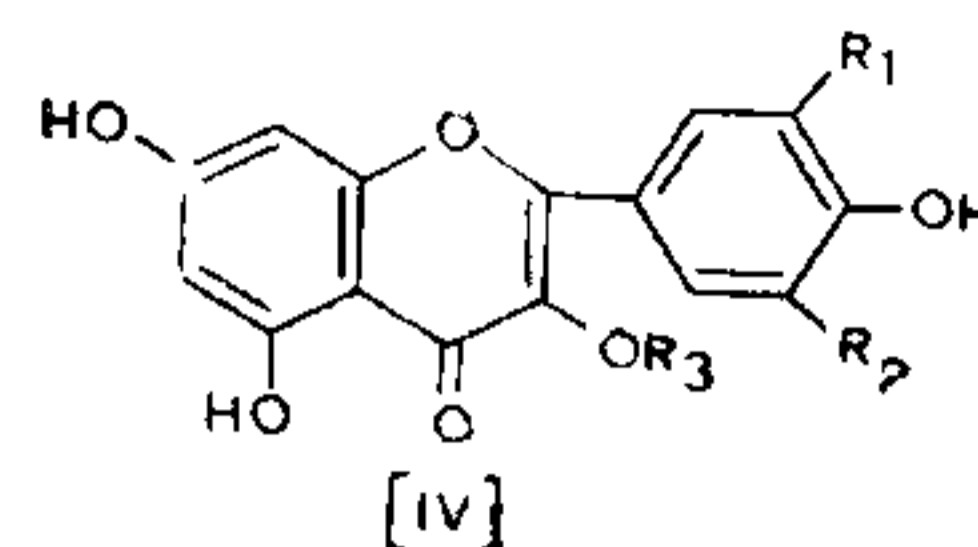
Flavanols and Their Gallates.—(i) Among the flavanols and their gallate esters could be listed as important (+) catechin (I a), (+) gallo-catechin (I b), (–) catechin (II a), (–) gallo-catechin (II b), (–) catechin gallate (II c), (–) gallocatechin gallate (II d), (–) epiafzelechin (III a), (–) epicatechin (III b), (–) epigallocatechin (III c), (–) epicatechin gallate (III d) and (–) epigallocatechin gallate (III e). Of these (–) epigallocatechin gallate (III e) and (–) epicatechin gallate (III d) predominated.



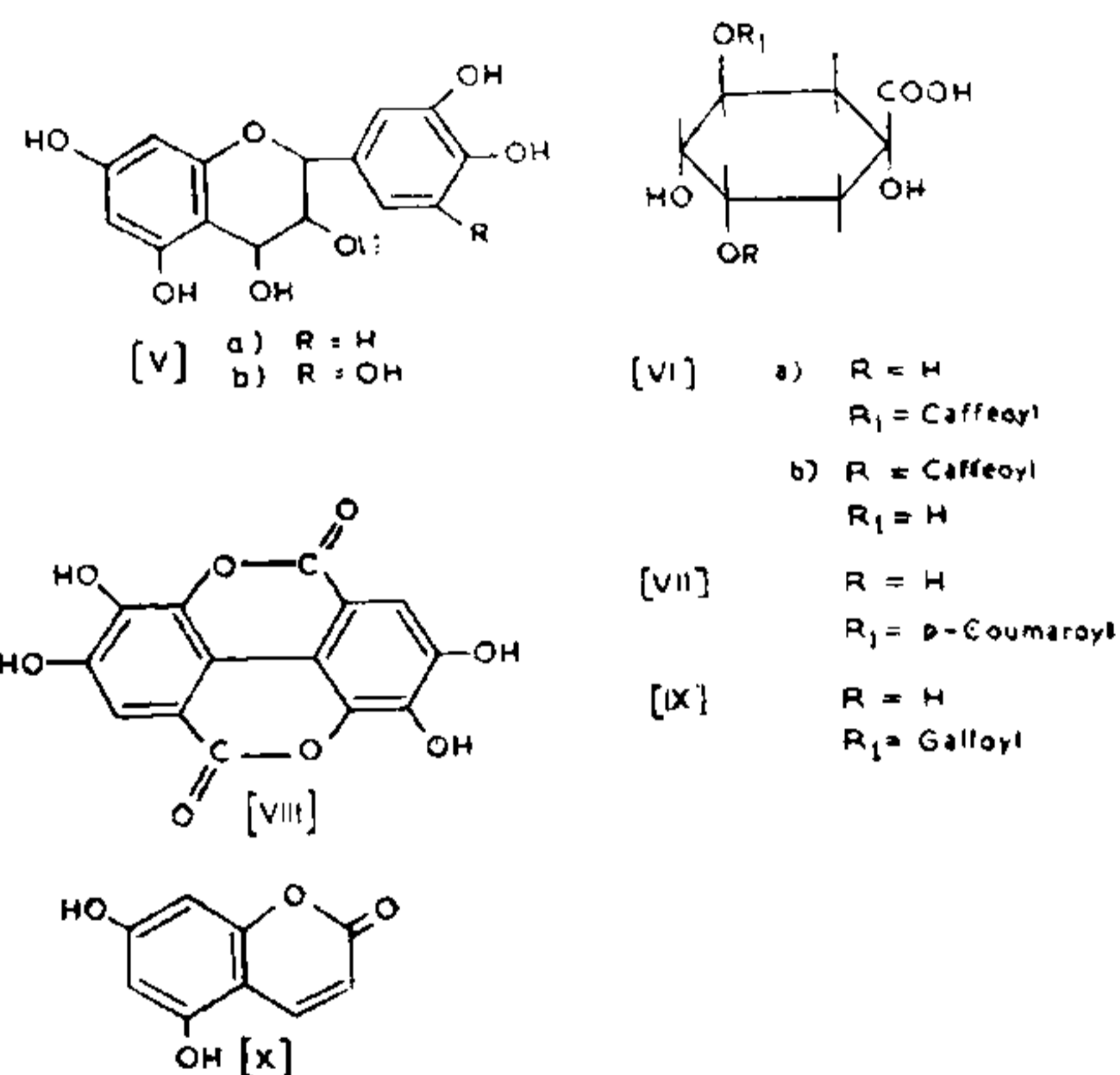
Flavonols and Their Glycosides.—(ii) Kaempferol (IV a), quercetin (IV b), myricetin (IV c), kaempferol-3-glucoside (IV d), kaempferol-3-rhamnoglucoside (IV e), kaempferol-3-rhamnodiglucoside (IV f), isoquercitrin (IV g), rutin (IV h), quercetin-3-rhamnoglucoside (IV i), myricetin-3-glucoside (IV j) and myricetin-3-rhamnoglucoside (IV k) and myricetin-3-galactoside (IV l) are the important compounds of the group of flavonols and their glycosides.

(iii) Among the leucoanthocyanidins, leucocyanidin (V a) and leucodelphinidin (V b) and the quinic acid derivatives, chlorogenic acid (VI a), neo-chlorogenic acid (VI b), p-coumarylquinic acid (VII), and theogallin (IX) have been identified.

Ellagic acid (VIII) and 5, 7-dihydroxycoumarin (X) are also reported to be present.



- (a) R₁ = R₂ = R₃ = H
- (b) R₁ = OH; R₂ = R₃ = H
- (c) R₁ = R₂ = OH; R₃ = H
- (d) R₁ = R₂ = H; R₃ = Glucosyl
- (e) R₁ = R₂ = H; R₃ = Rhamnoglucosyl
- (f) R₁ = R₂ = H; R₃ = Rhamnodiglucosyl
- (g) R₁ = H; R₂ = OH; R₃ = Glucosyl
- (h) R₁ = H; R₂ = OH; R₃ = Rutinosyl
- (i) R₁ = H; R₂ = OH; R₃ = Rhamnoglucosyl
- (j) R₁ = R₂ = OH; R₃ = Glucosyl
- (k) R₁ = R₂ = OH; R₃ = Rhamnoglucosyl
- (l) R₁ = R₂ = OH; R₃ = Galactosyl



Studies on the Variations in the Catechin Content.—Studies relating to the catechin content (generally referred to as tannin content) of the several varieties of Indian, Chinese and Japanese teas have been made¹. Teas of Indian origin showed the highest total catechin content, Chinese varieties being second and Japanese the least. The Indian teas were rich in the gallate esters and lowest in the simple catechins. Within the Indian varieties a comparative study was made and the Assam tea leaf had the highest content of tannins, catechins and total extractable substances². There was no qualitative difference among the different varieties; only quantitative differences were observed. The tea leaves from Assam and China were found to be very similar biochemically but different morphologically³.

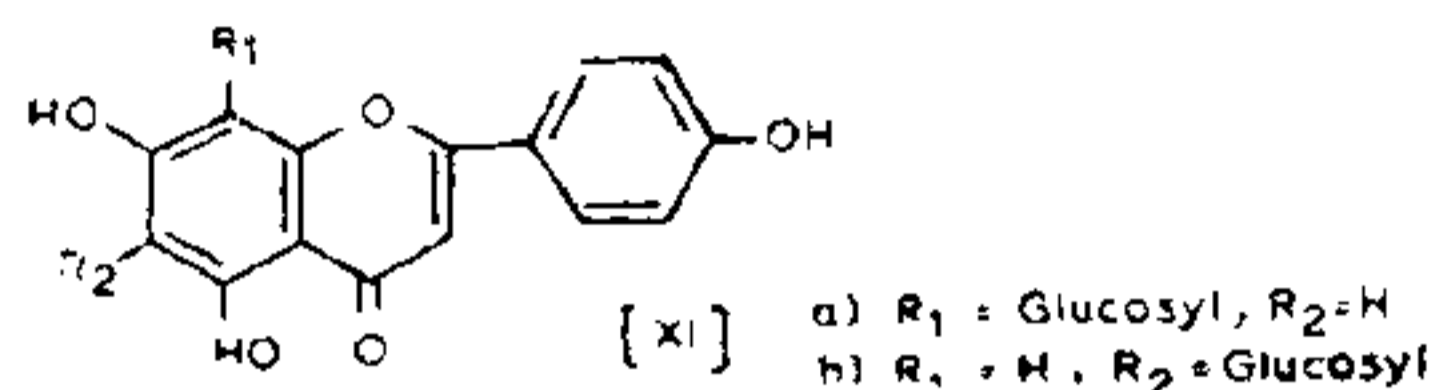
The content of catechins was higher in two-leaf shoots than in three-leaf shoots; the concentration in the buds was 20%, in the first leaf 17%, second 16% and third 12.5%⁴. An examination of Japanese green tea indicated the presence of (-) epicatechin, (-) epigallocatechin and their gallates but no (+) catechin or (+) galocatechin as found in Ceylon tea⁵. Variation of catechin contents with season during the growth of the tea leaf was also noted⁶.

Method of Study.—Two-dimensional paper chromatography was extensively used for the identification of flavanols.⁷ The application of circular paper chromatography for the separation of flavanols was found to be successful and the catechins were obtained in crystalline form⁸. The catechins and their gallates have also been separated by chromatography over Sephadex^{9,10}.

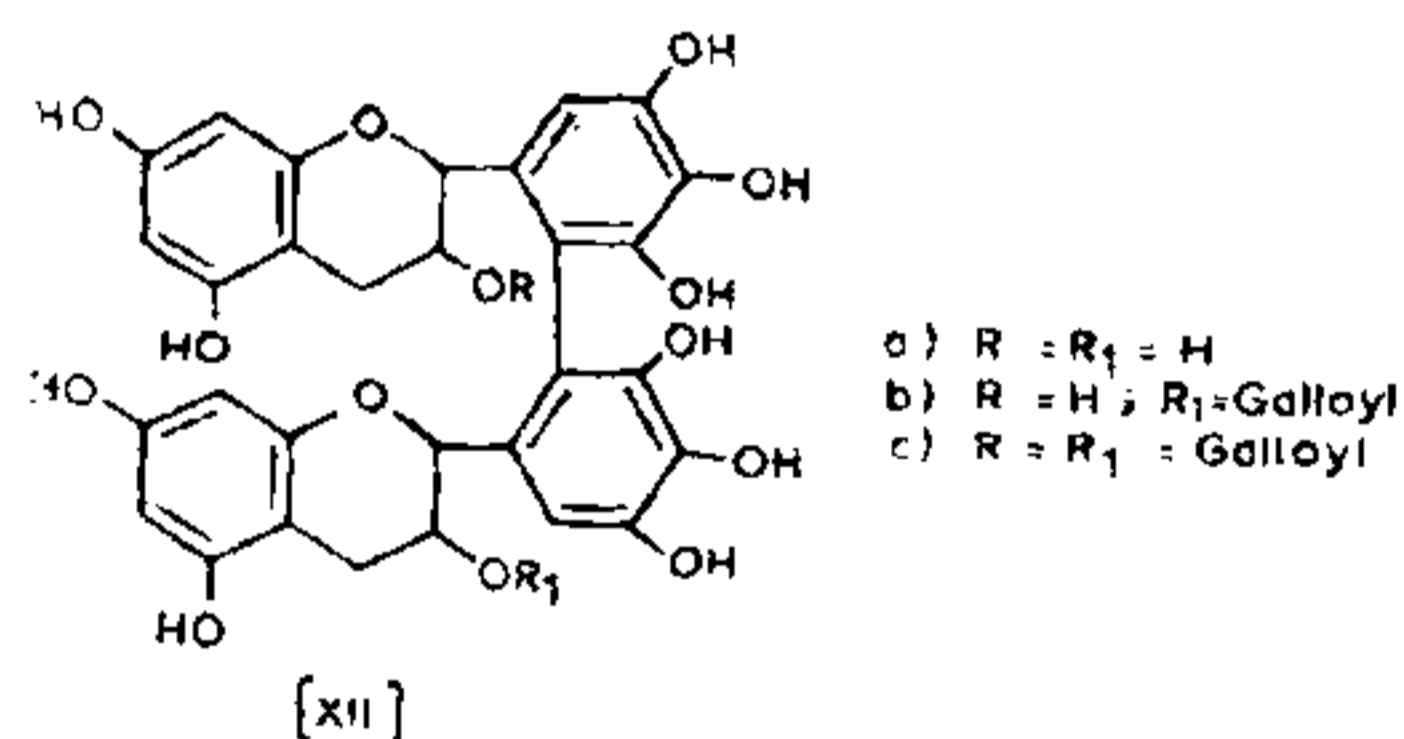
Flavonol and Flavone Glycosides and Their Changes.—As mentioned earlier the glycosides of myricetin, quercetin and kaempferol were found in tea leaves; they were also identified in green tea by two-dimensional chromatography and they could be separated by this technique¹¹. They were found to be present in black tea also¹². But there was a marked fall in their content compared to green tea. Conversion of these flavonols during the production of black tea was studied using commercial samples¹³. They were determined by reaction with boric acid in the presence of citric acid in various stages of tea production. The flavonol content was found to decrease gradually. Black tea contained 15–20% less than the green leaves. The content of myricetin glucosides underwent the greatest change. The flavonol triosides were less in the intermediate and final products than in the initial leaves. Flavonol loss was therefore not necessarily associated with the occurrence of free flavonol aglycones. The flavonols and their glycosides are regarded as the important constituents responsible for the yellow colour of black tea infusion.

Flavone glycosides have also been identified in green tea¹⁴. These glycosides belong to the special type known as C-glycosides. They are stable and are not hydrolysed by acids or enzymes and they are water soluble. They are, therefore, considered to be important constituents related to the colour of tea infusion. Three pigments in small quantities along with 15 minor components were isolated by preparative paper chromatography. Spectral studies indicated that these pigments belonged to the apigenin family. One of them was purified and obtained as an amorphous powder. The spectral and chemical evidences identified it

as 5, 7, 4'-trihydroxyflavone derivative. It is reported to differ from the known C-glycosylflavones in properties like melting point and solubility. This pigment seemed to be important for the colour of green tea as it gave a deep greenish yellow aqueous solution. There is also another report of the occurrence of 19 flavone glycosides in tea infusion¹⁵ detected by paper chromatography. Vitexin (XI a) and isovitexin (XI b) have been found in the mixture. Their UV spectra and colour reactions suggested that all of them are very closely related and all are C-glycosylflavones.



Polymeric Polyphenolic Compounds in Made Tea.—The flavanols present in fresh tea leaves are converted into different types of polymeric compounds during black tea manufacture by the action of an enzyme present in plucked tea leaves. The polymeric compounds include three bis-flavanols (XII a), (XII b) and (XII c) which were initially detected paper chromatographically by Roberts⁷ and later on obtained by cellulose column chromatography.

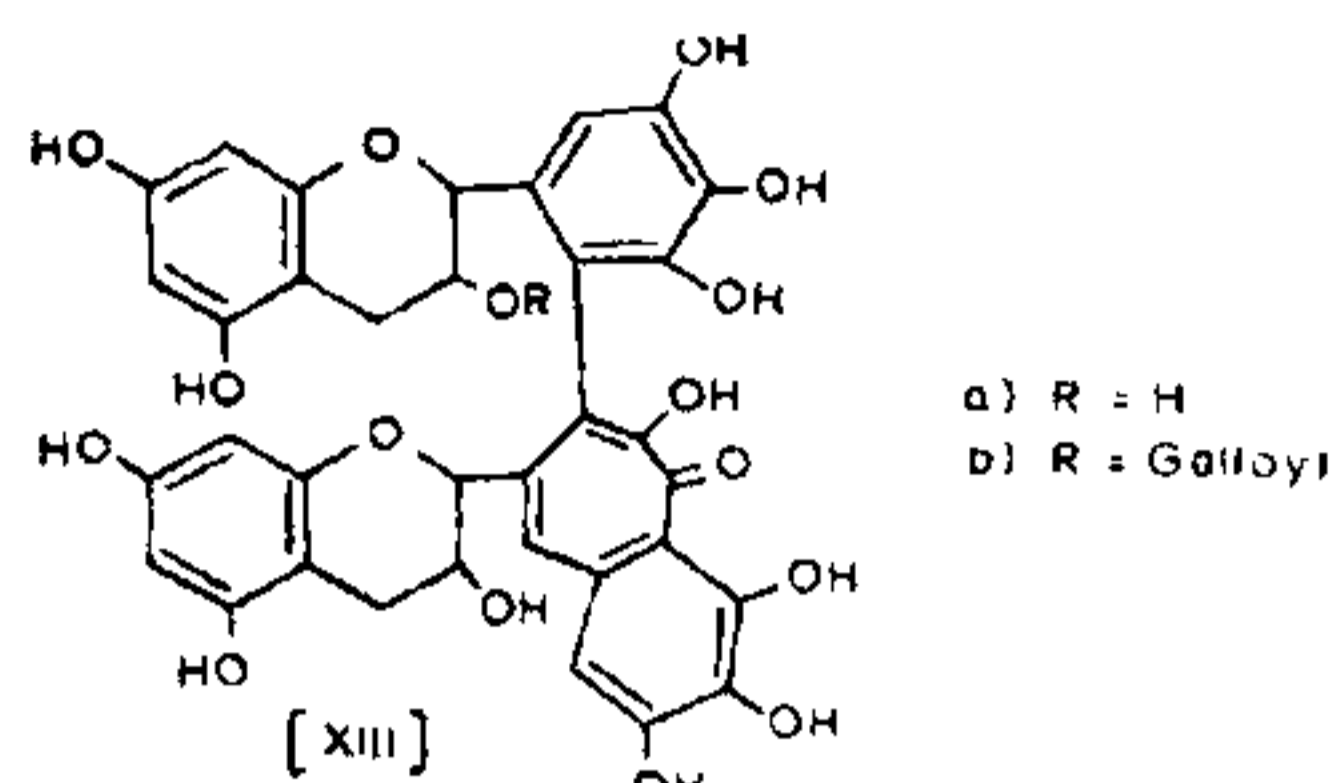


The coloured compounds fall under two important categories; (i) theaflavin, its isomers and theaflavin gallates and (ii) a complex brown mixture called thearubigins. Recently a few more members in the group of theaflavins have been obtained. They are discussed in the succeeding sections.

PART III. THE CONSTITUTION OF THEAFLAVINS

Development of Ideas Regarding the Structure of Theaflavins.—During the fermentation of black tea many chemical changes of importance take place. Roberts reported that catechins originally present in the tea leaves are oxidised during the fermentation process of tea manufacture to form coloured substances and named them theaflavins (yellow) and thearubigins (red). These two pigments have been considered to be important in determining the quality of tea. He thought that epigallocatechin and its gallate are the only polyphenols affected

during the fermentation process and suggested that theaflavins originated from the coupled oxidation of epigallocatechin and its gallate. He recognised the presence of a benzotropolone system in the nucleus and with the available limited evidences proposed the following structure (XIII a) for theaflavin and (XIII b) for its gallate⁷.

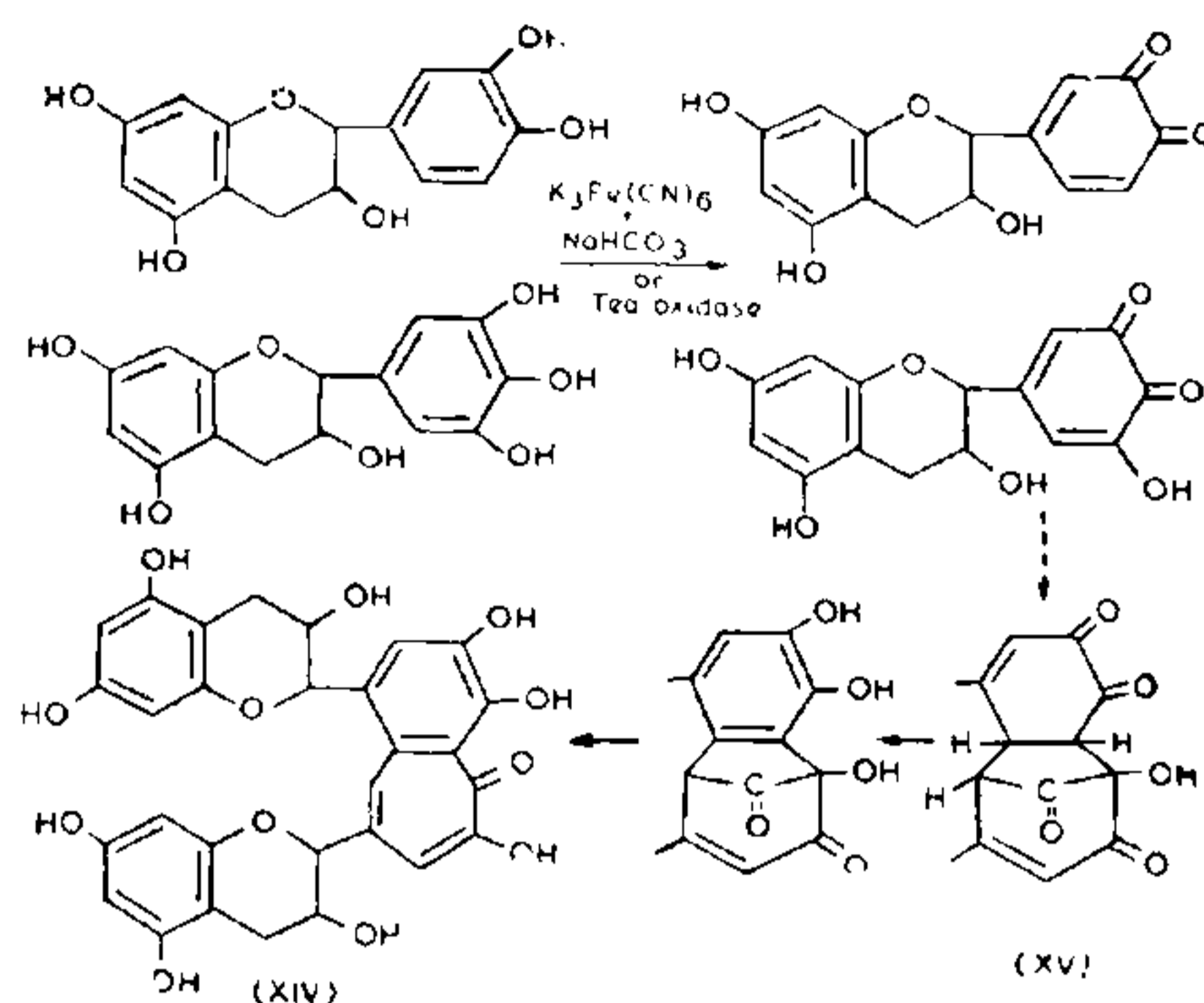


Later, Bhatia and Ullah observed a decrease in the content of epicatechin and also of its gallate during the fermentation process; hence it was concluded that they too should be participating in the above oxidation¹⁶. As the tentative structural formulae assigned to theaflavin and its gallate were not definite, a detailed study of the oxidation products of individual catechins and related compounds was taken up by Takino and co-workers.

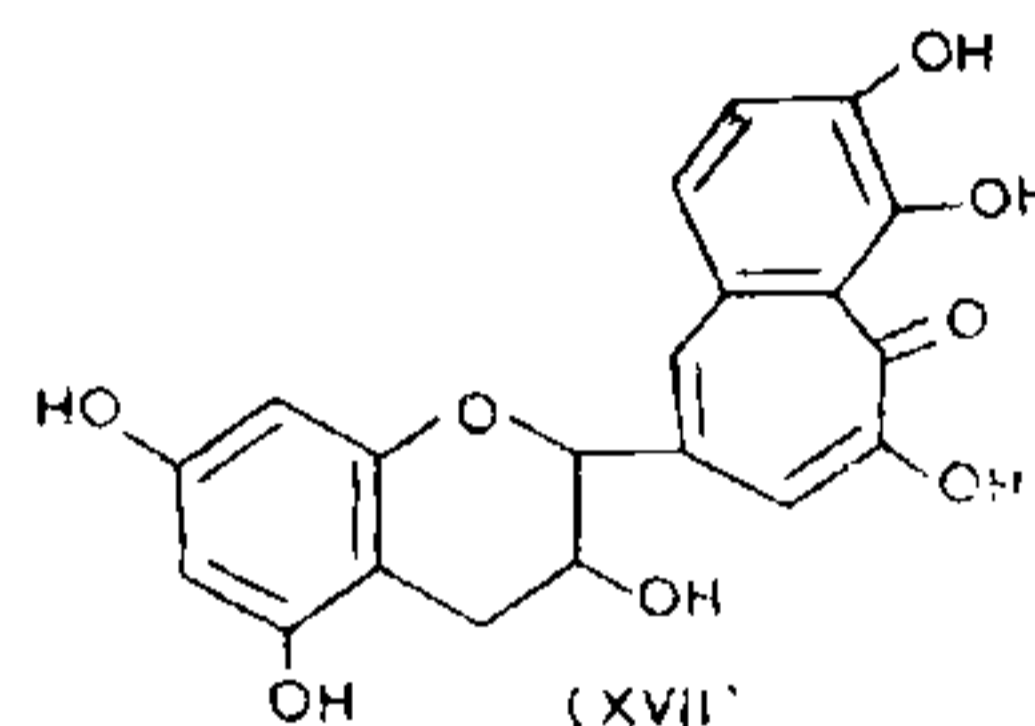
Synthetic Work Directed Towards the Understanding of the Structure of Theaflavins.—Takino *et al.* carried out the oxidation of a mixture of two substrates having different hydroxylation patterns: (i) vic-trihydroxy phenolic compound (having pyrogallol system) and (ii) an ortho diphenolic compound (having catechol system)¹⁷. When epigallocatechin alone was incubated with tea oxidase the product was found to be a simple colourless dimer (XII a). The addition of epicatechin resulted in the formation of a new orange coloured product. The same product was formed when the oxidation was carried out in a non-enzymic way by the use of alkaline potassium ferricyanide. Since the addition of epicatechin to the reaction mixture is necessary for the formation of the new pigment it was proposed that the reaction involved oxidative condensation of the two catechins resembling the oxidation of a mixture of pyrogallol and catechol to form 3', 4'-dihydroxybenzotropolone^{17a}. On this basis a tentative formula was assigned for this pigment and the following reaction scheme was formulated.

This synthetic pigment (XIV) was found to have the same properties as described for theaflavin by Roberts. It was identical with the ethyl acetate soluble fraction of black tea¹⁸. It was also observed that most of the theaflavin pigment in black tea was present as the gallate ester. On its treatment with the mould *Aspergillus niger* which

hydrolysed the gallate group only one product was obtained which was found to be identical with the synthetic pigment. The presence of theaflavin

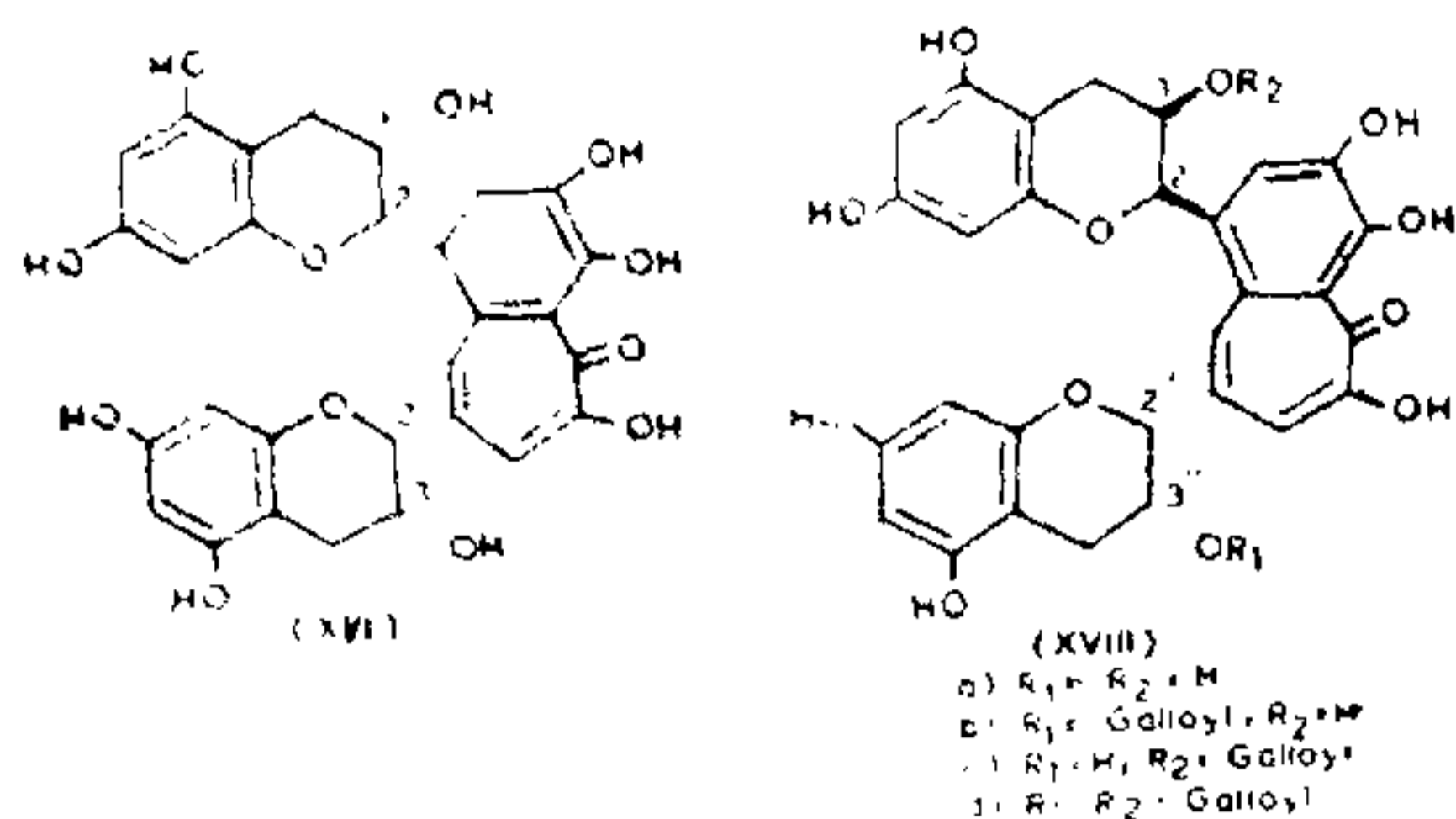


gallate in black tea has also been proved by its formation in the oxidation of a mixture of epigallocatechin gallate and epicatechin. The structure is further supported by the close resemblance of the UV and IR spectra of theaflavin with a substance called 'categallin' (XVII) produced by the oxidative coupling of epigallocatechin and catechol.

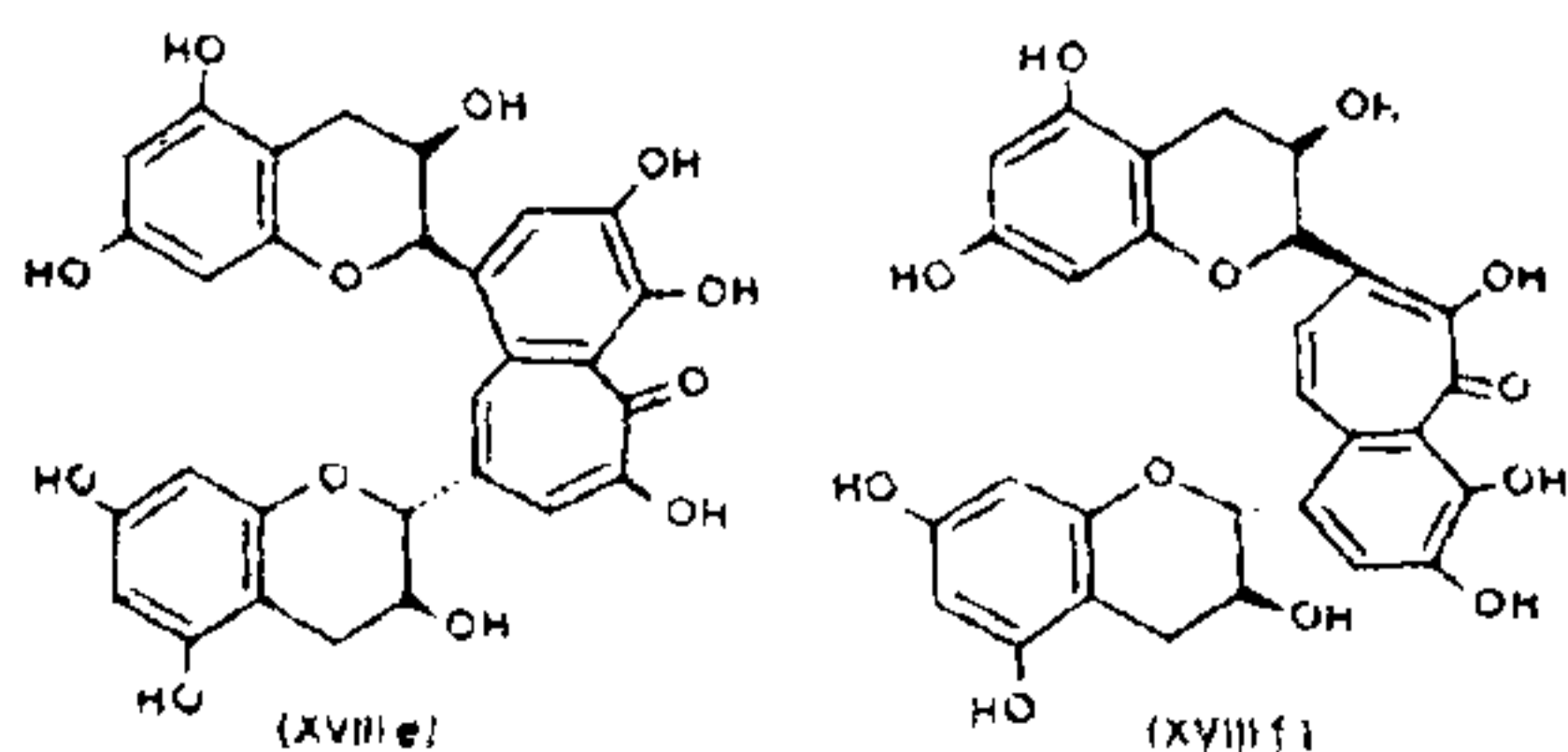


Configuration of the Flavan Units of Theaflavin.—The structure of theaflavin has been supported by NMR and mass spectrometric data¹⁹. From the NMR spectra of theaflavin, (+) catechin and (−) epicatechin and the relative position and splitting pattern of 2 and 2'' proton signals in theaflavin it was suggested that the pyran rings of theaflavin have the epicatechin stereochemistry. Later Brown *et al.* provided a more conclusive evidence for the proposed constitution of theaflavin²⁰. They suggested a configurational formula (XVIII a) different from the one suggested earlier (XVI) which was based on the assumption that the formation of theaflavin takes place with the preservation of absolute configuration at the chiral centre in the precursors. But in view of the possible epimerisation at C-2 of the catechin series, the consequences of this change during the formation of theaflavin from (−) epicatechin and (−) epigallocatechin was also considered. The benzylic protons at positions 2 and 2'' appeared as singlets in the NMR spectrum

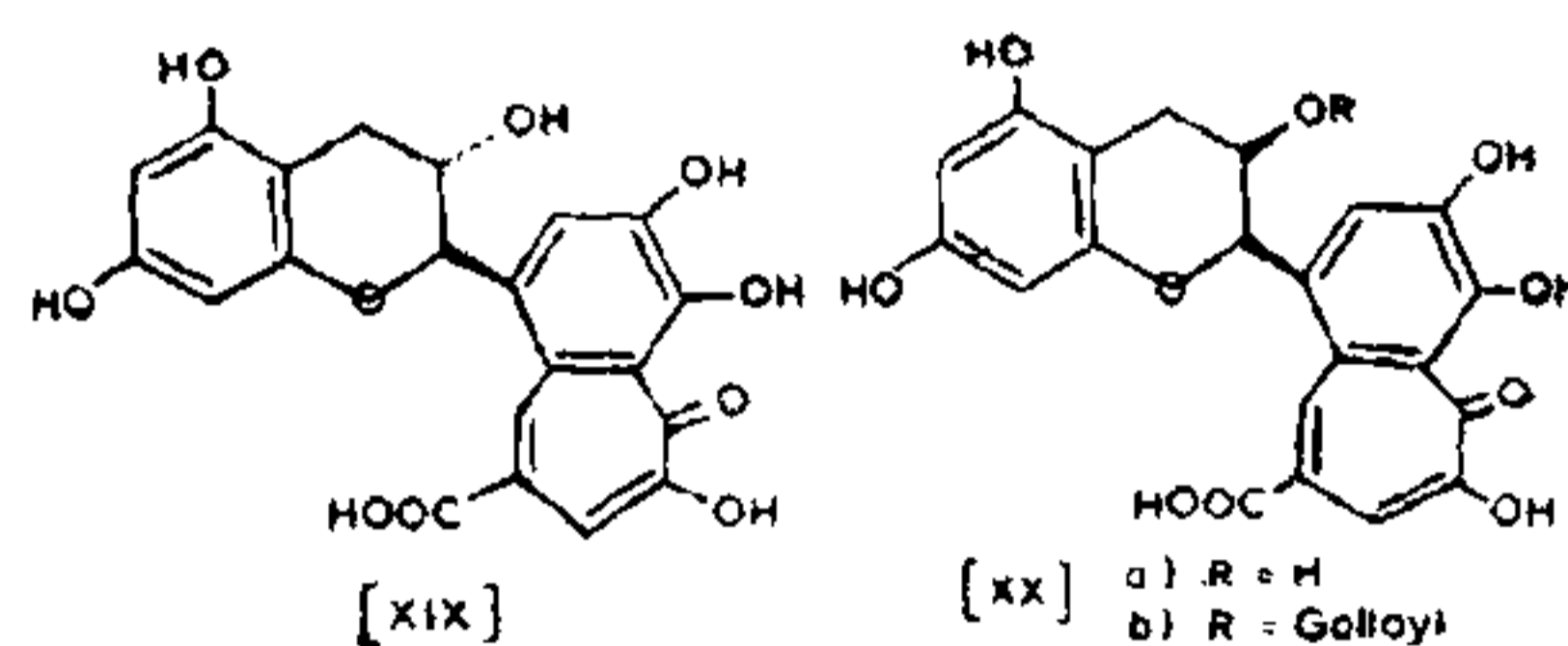
of theaflavin. This behaviour required axial-equatorial orientation (between C_2 -H and C_3 -H and C_2' and C_3' -H) resulting from *cis*-stereochemistry.



Identification of New Members of the Theaflavin Group.—Recently Ollis and his co-workers have reported the isolation and structure determination of the galloyl esters of theaflavin (XVIII *b*), (XVIII *c*) and (XVIII *d*) by spectroscopic methods²¹. They confirmed their structures by synthesis starting from theaflavin heptamethyl ether and trimethyl galloyl chloride. As it was considered that the enzyme catalysed oxidative couplings may not be specific, a search for the isomers of theaflavin was made and actually an isomer named isotheaflavin (XVIII *e*) was isolated. Since the chromatographic behaviour of isotheaflavin was similar to that of theaflavin gallates, the iso-compound could not possibly be identified earlier. The IR and UV spectra of theaflavin and isotheaflavin were very similar but NMR spectra showed some significant differences. The mass spectral fragmentations of theaflavin and that of isotheaflavin heptamethyl ether were similar thereby indicating that isotheaflavin is a diastereoisomer of theaflavin. It was considered to be the condensation product of (–) epicatechin and (+) gallocatechin. From a study of the coupling pattern of the two sets of 4 protons H^2 , H^3 , H^4 , H^{4*} associated with the rings of flavan-3-ol the structure of isotheaflavin was arrived at²². Recently there is another report of the isolation and characterisation of another isomer of theaflavin (XVIII *f*) by spectral data and also by synthesis^{23a}. It was shown to be formed by the condensation of (–) epigallocatechin and (+) catechin.



Three more components have been isolated in pure form and named 'theaflavic acid' (XIX) and 'epitheaflavic acid' (XX *a*) and epitheaflavic acid-3'-gallate (XX *b*). These pigments have been identified as the condensation products of (+) catechin and gallic acid, (–) epicatechin and gallic acid and (–) epicatechin gallate and gallic acid respectively. The structures have been confirmed by spectral data and also by synthesis^{23, 23a}.

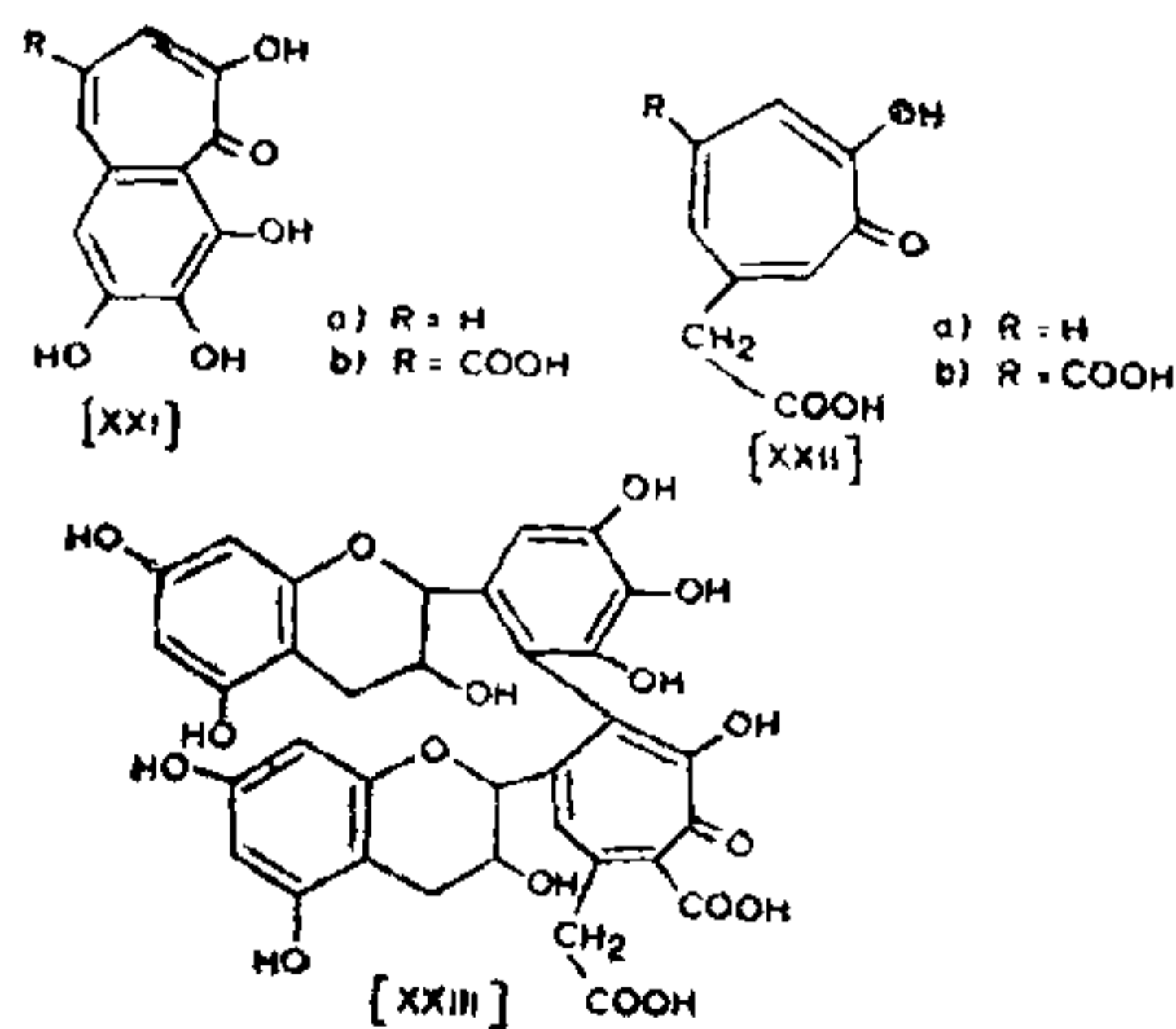


Theaflavin formation involves an enzyme catalysed process during the fermentation process of black tea manufacture. Its novel C_{29} structure is derived by oxidative coupling of C_{15} flavonoid precursors and can be regarded as a new variant of the C_{30} -structures characteristic of the biflavonoids and the dimeric proanthocyanidins.

PART IV. THE CONSTITUTION OF THEARUBIGINS

Earlier Proposals for the Structure of Thearubigins.

—The thearubigins constitute about 10–20% by weight of black tea⁷. They have been examined in several laboratories, but there was no agreement about the class of natural products to which they belonged. Roberts suggested that the acidity of thearubigins could be due to the presence of carboxyl groups. He considered the thearubigins to be the oxidation products of theaflavins and envisaged the destruction of the benzene nucleus thereby leaving behind the tropolone part in the process. He proposed this in analogy with the oxidation of purpurogallin (XXI *a*) and purpurogallin carboxylic



acid (XXI *b*) to yield 2-carboxy and 2, 6-dicarboxy derivatives of 4-hydroxy-3-keto-cycloheptatrienyl acetic acid (XXII *a*) and (XXII *b*) respectively. On these considerations the following structure (XXIII) was proposed.

Using aqueous ethanol (80%) for extraction, Vutz and Brandenberger concluded that thearubigins are partly composed of substances related to humic acids. They also indicated the presence of nitrogen in the thearubigins to the extent of 0.55% which could not be accounted for by caffeine alone. The identification of fourteen amino acids obtained by acid hydrolysis of thearubigins was also recorded leading to the suggestion that the *o*-quinones formed out of 3', 4'-dihydroxy and 3', 4', 5'-trihydroxy-flavone derivatives being reactive condensed with amino acids or proteins yielding humic acid-like substances. Wickremasinghe has described investigations at the Tea Research Institute, Ceylon, regarding an association of the thearubigins with chebulinic acid, corillagin and glucogallin which have been earlier obtained from tannins. Millin and Rustidge have referred to thearubigins as a complex mixture of predominantly acidic substances varying in molecular weight in the range 700–40,000 or more. They also expressed the view that the thearubigins may be produced by the interaction of *o*-quinones with the components of the leaf other than polyphenols but no further evidence was presented by them regarding their chemical nature²⁴.

Recent Developments on the Structure of Thearubigins.—Subsequently Ollis and co-workers have examined thearubigins using Roberts' isolation methods^{24, 25}. The nitrogen associated with these fractions has been shown to be present as caffeine and the caffeine could be removed either by direct extraction with chloroform of each fraction or by prior extraction of the tea liquor with chloroform.

Successive extraction of an aqueous extract of black tea was made with chloroform, ethyl acetate and *n*-butanol. The chloroform extract yielded mainly caffeine. The other two extracts were purified by dissolving the residue in acetone and precipitating with chloroform, dissolving the precipitate in acetone and precipitating with ether and finally dissolving the precipitate in methanol and precipitating with ether. Five main fractions could be obtained and they showed similar behaviour. The chemical and chromatographic behaviour of these five fractions suggested that their constituents belonged to one general structural type; hence instead of further separating each mixture, selective degradative experiments were carried out on each one of the five fractions. The following reactions

and observations gave the clue for the understanding of the nature of thearubigins.

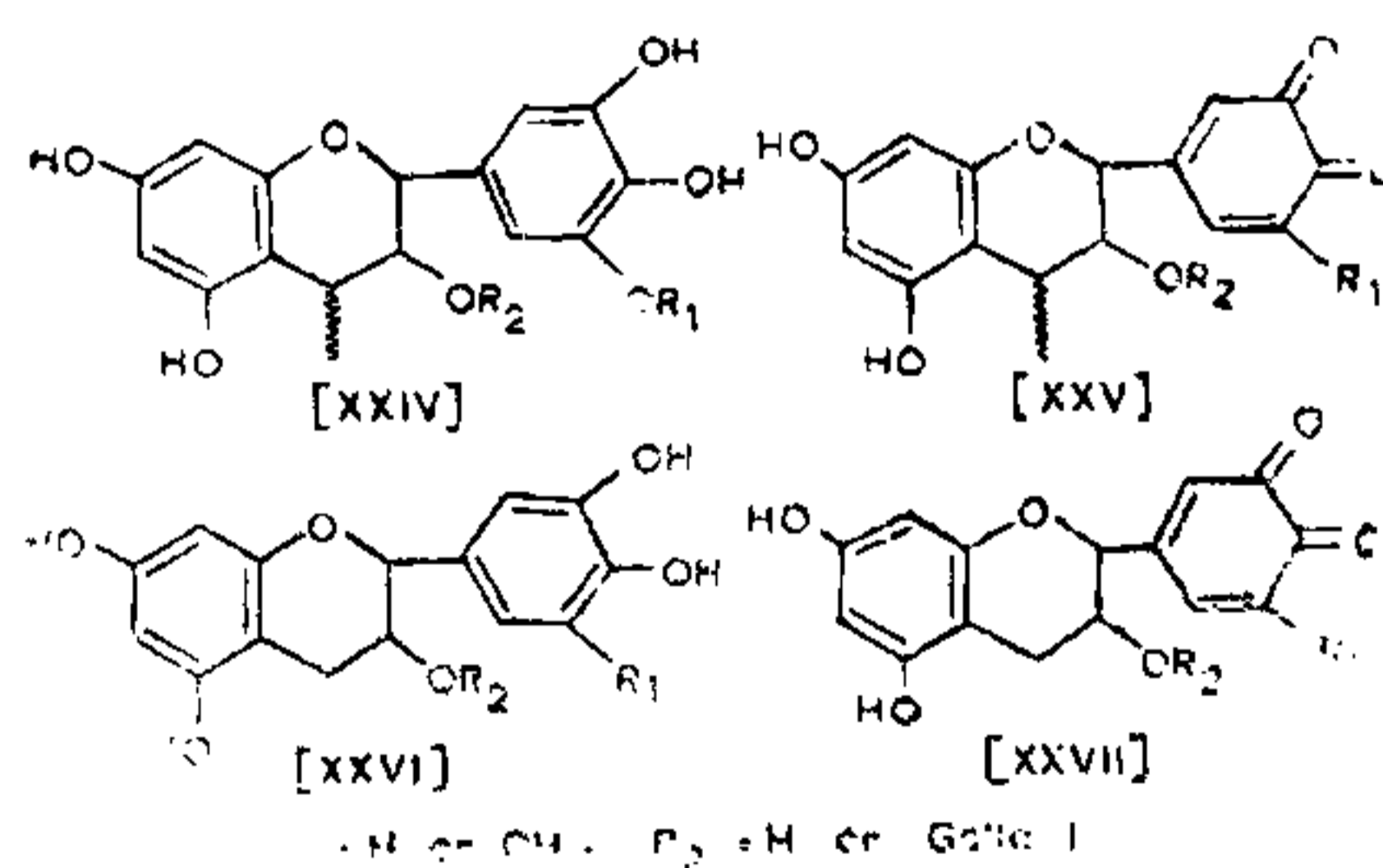
(i) Hydrolysis with 0.035 N HCl in anhydrous isopropanol yielded cyanidin (V *a*), delphinidin (V *b*) and their isopropyl derivatives. They were identified chromatographically by comparison of their R_f values in various solvent systems.

(ii) Treatment in aqueous ethanol with sulphur dioxide yielded (+) catechin, (–) epicatechin, (–) epicatechin gallate, (+) gallo catechin and (–) epigallocatechin gallate but not all of these were isolated from each thearubigin fraction.

(iii) The presence of gallate esters was also confirmed by the isolation of gallic acid on acid hydrolysis of thearubigins.

(iv) The IR spectra of the methyl ethers of thearubigins indicated the presence of alcoholic hydroxyl groups. The methyl ethers could be acetylated. These results suggested the presence of phenolic and alcoholic hydroxyl groups in thearubigins.

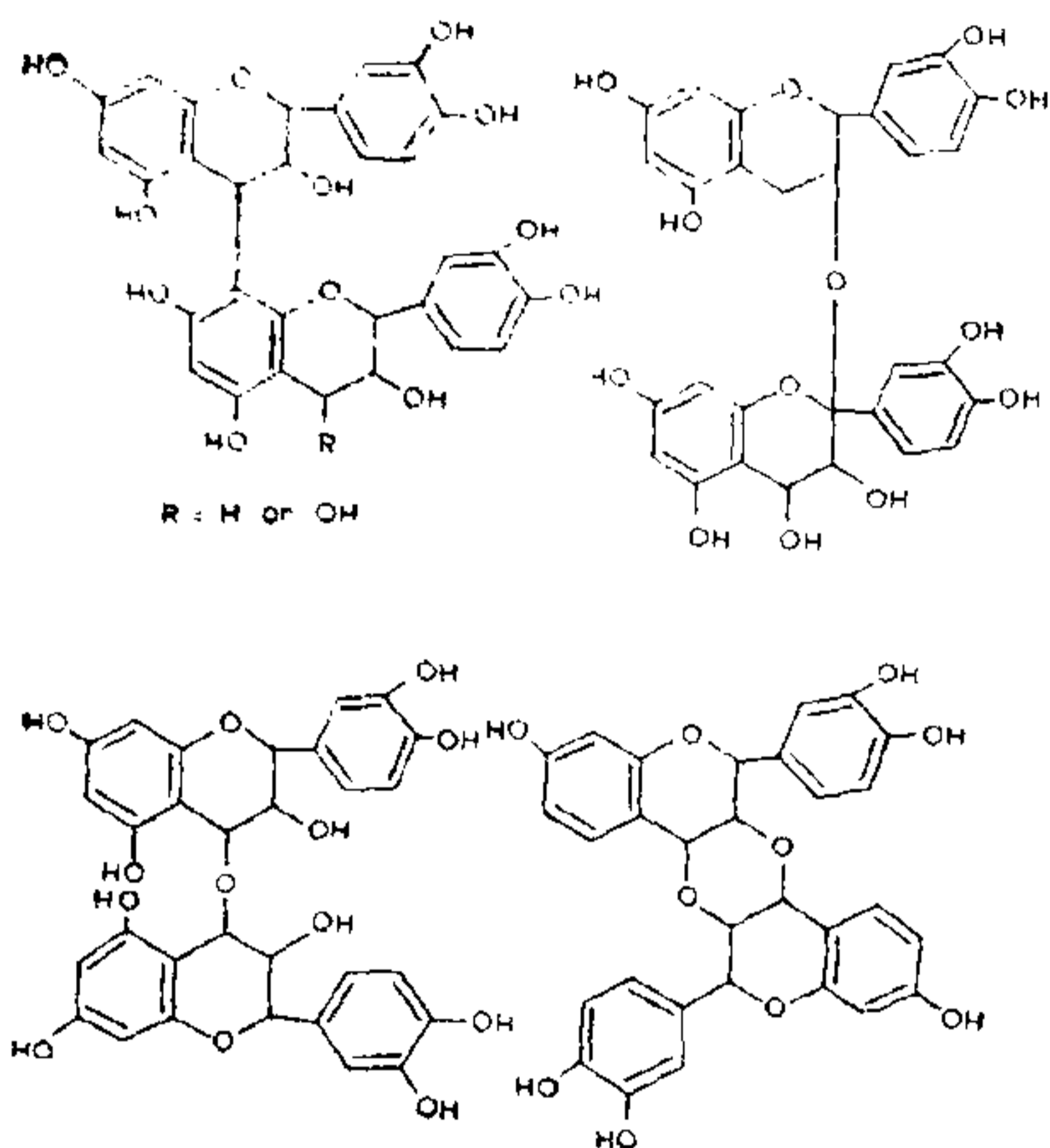
(v) Colour and spectral data of the thearubigins suggested the presence of *o*-quinonoid residues. This was evidenced by reduction with sodium dithionite. It was inferred that two types of *o*-quinonoid residues associated with ring B of the flavanoid units (XXIV) and (XXVI) could be visualized and the acidity of the thearubigins could be explained satisfactorily as due to the presence of hydroxy orthoquinone residues (XXV) and (XXVII).



The wide occurrence of polymeric proanthocyanidins is well known and the chemistry of these compounds has been well studied²⁶. Some typical structures of proanthocyanidins are given below. Some of them have catechin units. The results obtained in the above experiments coupled with the properties of these types of compounds known earlier have led Ollis and his co-workers to suggest that thearubigins belong to proanthocyanidin class of compounds.

The Possible Linkages in Thearubigins.—The isolation of two types of products from the reductive

hydrolysis, that is, the catechins and the sulphur containing compounds which yield cyanidin and delphinidin on acidic treatment indicates a minimum of two types of bonding in the polymeric proanthocyanidin structure. By bond cleavage reactions at 4 position of the flavanoid residues sulphur-containing compounds could arise whereas catechins could be envisaged to arise in the reductive hydrolysis from flavanoid residues which are not bonded *via* position 4. However the individual polymeric entities have not been isolated; hence the evidence for the interflavonyl linkages is only circumstantial.



Some compounds of the thearubigins type are not extracted from aqueous solution by organic solvents. They are considered to be substances of polyphenol protein type in which the polyphenol concerned is a polymeric proanthocyanidin.

Recently Sanderson and co-workers studied the oxidation of (-) epicatechin and its gallate separately with gallic acid and obtained epitheflavic acid (XX a) and 3-galloylepitheflavic acid (XX b). They also observed that the presence of (-) epicatechin helped the transformation of epitheflavic acid into thearubigins. These results possibly give clues for the formation of thearubigins in black tea.

PART V. METABOLISM OF FLAVANOLS OF TEA PLANT

Among the polyphenolic constituents of tea leaf, six flavanols are found to form a substantial proportion of the total polyphenol content. The earlier work on the estimation of these compounds at various growth stages indicate the gradual lowering of catechin content (measured by the Lowenthal method) during the development of leaves. Fur-

thermore the influence of seasonal changes on the increase of catechin content, e.g., from spring to mid-summer have been observed. This study indicated a considerable variation of individual flavanol content such as the greater predominance of galloyl products in the younger parts of shoots and during mid-summer. These results have been supported by the work of Nakagawa and Torii²⁸. In general, younger shoots were rich in catechin gallates and poor in free catechins as compared to more developed ones. Stems were distinctly poor in flavanols except (-) epicatechin. The samples of the second or third season (in mid-summer) contained more flavanols than those of the first (in spring), gallates especially accumulating in mid-summer.

The earlier work on the variation in catechin content has generally found a gradual decrease with leaf growth. This fall is actually due to the variation in the relative proportion of flavanols in this investigation. Methods usually employed for determining total (catechin) content, such as Lowenthal's method, estimate the number of pyrogallol groups in the molecule. Therefore a decrease in the proportion of galloyl flavanols causes a consequent lowering of the total catechin value. By comparing the values of galloyl and total flavanols determined by paper chromatographic and Lowenthal methods, it can be seen that the values of tannins in leaves correspond approximately to those of galloyl flavanols.

Further it was found that there were increases of (+) catechin, (+) gallocatechin and certain flavanols during the development of leaves. Hence it may be concluded that the most characteristic figures of variation with growth are the decrease of catechin gallates and the increase of catechins so long as the shoot is developing or the leaf is expanding.

PART VI. PHARMACOLOGICAL PROPERTIES OF BLACK TEA POLYPHENOLS

Among the numerous types of compounds that come under the category of polyphenols the flavonoids have attracted the attention for over three decades as Vitamin P components which are capable of keeping the blood capillaries efficiently functioning. Many of them are used clinically. A more recent development is the importance of the size of the polyphenol. Biflavonoids have been found to have specially useful properties²⁹. Since theaflavins and thearubigins could be considered as the structural variants of biflavonoids we carried out the pharmacological assays and found that they have pronounced bradykinin antagonism and anti-spasmodic action against prostaglandins³⁰.

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STRAIN SELECTION IN CITRIC ACID FERMENTATION*—A REVIEW

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A NUMBER of organic acids are produced by moulds and bacteria through the process of fermentation, and citric acid is one of the most important metabolic products now produced commercially by fermentation with specific moulds, mostly strains of *Aspergillus niger*.

Wehmer²⁹ was the first to report that citric acid was produced from sugar through fermentation by moulds named by him as *Citromyces pfefferians* and *C. glaber*. Later, many other fungi have been found

to ferment sugar to produce citric acid, but today some strains of *Aspergillus niger* are used for commercial production of citric acid in many countries and the problem has been attacked from diverse angles by various investigators. The present paper is intended to report the several techniques adopted by various investigators to improve the yield of citric acid in the laboratory.

When one is to improve the yield of a product elaborated by a microorganism, two general pathways are open: (a) improvement of the parent strain and (b) improvement of the environmental condition. That means, an organism capable of synthesizing more of the compound in question is of prime importance, which should then be studied

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