Enzymic Oxidation of Catechin to a Polymer Structurally Related to some Phlobatannins

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The formation of polymers and hydrogen peroxide during the autoxidation at 35° and pH 6–8 of catechin and of related 3′:4′-dihydroxyflavans has been studied by measurement of oxygen uptake, and by the elementary analyses, absorption spectra and reactions of the dialysed polymers (Hathway & Seakins, 1957*a*). The evidence obtained, together with that provided by the spectroscopic study of intermediates produced by silver oxide oxidation (Hathway & Seakins, 1955), supports the quinone polymerization mechanism for catechin autoxidation. Whereas oxidative coupling of 5:7-di-*O*-methylcatechin resembles that of catechol, quinone polymerization of catechin and of 5:7:3′:4′-tetrahydroxyflavan involves the phloroglucinol residue.

The mechanism for the autoxidation of catechin leading to a recognizable polymer has now been reported (Hathway & Seakins, 1957*a*), but as there was little information relating to the enzymic oxidation of catechin and no information concerning the product (Roberts & Wood, 1950), the aerobic oxidation of catechin to a polymer precisely similar to the autoxidation polymer has now been investigated by using different plant polyphenoloxidases. Since *Uncaria gambir* Roxb. (Rubiaceae) leaves and *Acacia catechu* Willd. (Leguminosae) heartwood are known to contain large quantities of catechin and its epimeride respectively, the tannin extractives of these plant tissues have been examined for the occurrence of phlobatannins related to the polymers from the autoxidation and polyphenoloxidase oxidation of catechin. It should, however, be stated that as these phlobatannins were isolated from harvested plant materials, they may not be localized within living tissues of the plants, but may have arisen subsequently by enzymic or chemical change or both. This possibility does not affect the work to be described, which is concerned with the biogenesis and structure of the phlobatannins produced under the conditions encountered. In general, plant phenolics, including phlobatannins or their precursors which originate in the cambium, migrate into the dead heartwood and bark tissues (Erdtman, 1956; Hillis, 1956; Jaccard, 1938). Abscission stimulates phlobatannin formation in a seasonal crop such as leaves (Forsyth, 1952*b*; Goris, 1907; Roberts, 1952). The plants with which this work is concerned are valued for their phlobatannin extractives.

EXPERIMENTAL

General. Evaporations were carried out in N_2 under reduced pressure at <35°. Paper chromatography was carried out at 23 ± 2° in all-glass apparatus. Chromatograms were dried at room temperature, unless otherwise stated. A Hanovia mercury-arc lamp fitted with a Wood's-glass filter was used to examine chromatograms for fluorescent zones. Hide-powder tests were made by the method of Grassmann, Endisch & Kuntara (1951).

Acetone-dried powders of Psalliota campestris *and* Nicotiana tabacum. Freshly picked mushrooms (350 g.) were plunged into ice-cold acetone (2 l.), sliced, and homogenized in a top-drive macerator. The homogenate obtained was filtered through sintered glass and the solid (20 g.) was immediately washed five times with 500 ml. portions of ice-cold acetone, and dried at 0° *in vacuo*. This powder (2·8 enzyme units) had a polyphenoloxidase activity of purpurogallin number 0·14. The purpurogallin number (P.N.) was determined by the method of Keilin & Mann (1938) but purpurogallin was estimated in ethanol solution at 375 and 430 mμ by means of a standard u.v.-light spectrophotometer. One enzyme unit (E.U.) corresponds to the quantity of enzyme which produces 1 g. of purpurogallin in 5 min. at 20°.

The acetone-dried powder of *Psalliota campestris* was used without further treatment for enzyme-catalysed oxidations, whereas the acetone-dried powder of *Nicotiana tabacum*, P.N. 0·0024, was first eluted with ice–water (10 ml./250 mg.) until free from amino acids.

Potato juice. A chilled potato was minced and pulverized, and the juice expressed was filtered, chilled, and centrifuged to remove starch (Raper & Wormall, 1923). Freshly prepared juice had polyphenoloxidase activity of P.N. 0·0022, and was used immediately or after preliminary dialysis in Visking 18/32 seamless cellulose tubing.

Manometry. Use was made of Haldane's (1921) constant-pressure respirometer, in which the reaction vessel was connected by small-bore pressure tubing to a gas-burette and to a constant-pressure manometer, the other limb of which was connected to a compensation vessel. Both the reaction and the compensation vessels were shaken at 20° at a speed sufficient to maintain the reaction mixture as a foam. *n*-Butyl phthalate was used in the constant-pressure manometer and gas-burette.

Isolation of tannins from Acacia catechu *and* Uncaria gambir. A 5% (w/v) aqueous solution (400 ml.) of extract (containing 43% of the total solids as tannin, by hide-powder test) prepared from *Acacia catechu* heartwood was

extracted for 2 days with ether and then for 5 days with ethyl acetate. Progress of solvent extraction was followed by means of two-dimensional paper chromatography. For this purpose, samples (5 μl.) of the test solutions were spotted at a distance of 2 cm. from both edges of the lower left-hand corner of Whatman no. 1 filter papers, 25·5 cm. square, and chromatographed by the ascending method with N-acetic acid as first-way solvent and *n*-butanol–acetic acid–water (6:1:2, by vol.) as second-way solvent.

Extraction with ether was continued until catechin, R_F 0·54, 0·74, and *epi*catechin, R_F 0·49, 0·62, had been removed. The ether residue (6 g.) constituted 30% (by wt.) of the whole extract. In addition to catechin epimers this fraction contained flavonols and substances of unknown composition. Extraction with ethyl acetate was similarly continued until other substances of unknown composition had been removed. The ethyl acetate residue (4·6 g.) constituted 23% (by wt.) of the original extract. The residual aqueous solution contained 58% of the total tannin (determined by hide-powder test), and paper chromatography showed a single phenolic component at the origin. This tannin was precipitated from dilute solution either by 1% (w/v) gelatin reagent, or when refluxed with a mixture of formalin (40% formaldehyde) and 12N-HCl. In order to recover an analytical sample of this tannin from the residual aqueous solution, deionization was first carried out by electrodialysis. The aqueous solution was accordingly transferred to the middle compartment of a cell, constructed from Pyrex pipe-line components (Lovering & Smith, 1946), but fitted with a Permaplex A-10 anion-exchange membrane (The Permutit Co. Ltd., London, W. 4) and a Permaplex C-10 cation-exchange membrane. The apparatus was supplied with 100v d.c. until after 30 hr. the resistance approached a limiting value. Precipitation of tannin paralleled deionization. The supernatant was centrifuged off, and the sediment was washed with five successive 50 ml. vol. of water, and centrifuged after each addition and the supernatants were discarded. The phlobatannin, which was dried over P_2O_5 at 20° *in vacuo* for 3 days and at 70° *in vacuo* for 8 hr., did not give the ferric and vanillin reactions.

A 5% (w/v) aqueous solution (400 ml.) of extract (containing 40% of the total solids as tannin, by hide-powder test), derived from *Uncaria gambir* leaves by water percolation, was similarly extracted with solvents. The ether residue (9 g.) and the ethyl acetate residue (2·5 g.) constituted 45 and 12·5% (by wt.) respectively of the original extract. Catechin (8·6 g.) crystallized from a solution of the ether residue in aqueous acetone, forming needles, m.p. 176–177°, undepressed by admixture with an authentic specimen. The residual aqueous solution accounted for 65% of the total tannin (hide-powder test), and paper chromatography revealed a single phenolic component at the origin. This tannin was precipitated from dilute solution either by 1% (w/v) gelatin reagent, or by refluxing with a mixture of formalin (40% formaldehyde) and 12N-HCl. The phlobatannin which was recovered after deionization and elution as described above, was dried at 70° *in vacuo*, and did not give the ferric and vanillin reactions.

5-Methoxy-4-methylresorcinol. A specimen, m.p. 120° (Found: C, 62·1; H, 6·7. Calc. for $C_8H_{10}O_3$: C, 62·4; H, 6·5%), was prepared by the method of Robertson & Whalley (1951).

Separation of the flavonols from Acacia catechu *by paper chromatography.* Spots (10 μl.) of a 2% (w/v) solution of the ether residue in ethanol were applied to base lines, 4 cm. from the lower edge of five sheets of Whatman no. 3 filter paper 57 cm. long. Single-way ascending chromatography was effected with N-acetic acid. After the papers had been irrigated for 4 hr. they were dried and given a second development with N-acetic acid to ensure complete migration of the catechins. Transverse zones, containing the flavonols, were then located in u.v. light, and cut out, and extracted with boiling ethanol. Spots (5 μl.) of a 0·5% solution of the ethanol residue (3 mg.), and marker spots (5 μl.) of 0·5% solutions of fisetin, myricetin, quercetagetin, quercetin and robinetin respectively were applied to Whatman no. 1 filter papers, which were irrigated for 24 hr. by the ascending method with the following solvent systems: *m*-cresol–acetic acid–water (25:1:24, by vol.; upper phase) (Bate-Smith, 1949), and butan-2-ol–acetic acid–water (14:1:5, by vol.). Papers which had been chromatographed with the solvent system containing *m*-cresol were dried at 60°. Individual flavonols were detected by their fluorescence in u.v. light.

Isolation of quercetagetin from Tagetes erecta *flowers.* The occurrence of quercetagetin in *Tagetes erecta* flowers was observed by Mahal (1938), and the flavonol was therefore isolated from this source by the following simple procedure. Petals (40 g.) from the orange flowers were disintegrated in a top-drive macerator in 250 ml. of 95% ethanol, and the homogenate obtained was refluxed for 1 hr. The filtrate was concentrated to 20 ml. and refluxed with 100 ml. of 2N-H_2SO_4 for 2 hr., when a trace of insoluble material was removed. When residual solvent was evaporated from the aqueous phase, precipitation occurred, and the solid recovered was extracted consecutively with light petroleum (b.p. 40–60°, 800 ml.) and boiling ether (500 ml.). The petroleum extract was discarded. Dropwise addition of water to a solution of the ether residue (250 mg.) in 10 ml. of ethanol deposited traces of tar, which were removed. Further dilution of the filtrate precipitated quercetagetin, which was twice crystallized from ethanol, forming yellow needles (50 mg.), m.p. 310° (decomp.), $\lambda_{max.}$ 259 (log ϵ 4·28); 362 (log ϵ 4·31) mμ; inflexion at 272 (log ϵ 4·15) mμ (Found: C, 56·9; H, 3·4. Calc. for $C_{15}H_{10}O_8$: C, 56·6; H, 3·2%).

RESULTS

The present work consists of an exploratory study of the enzymic oxidation of catechin to a polymer, precisely similar to a polymer produced by autoxidation (Hathway & Seakins, 1957*a*) and comparable with phlobatannins which have now been isolated in high yield from amongst the tannin extractives of two plants (Hathway & Seakins, 1957*b*).

Crude enzyme preparations were used, since in many cases the polyphenoloxidase activity has been found to be identical with that for the more purified enzyme (Raper, 1932), and our early results also showed that greater difficulty in measuring the initial rate of oxidation was encountered with purified than with crude enzyme preparations.

During the aerobic oxidation of catechin by mushroom polyphenoloxidase at 20° and pH 8, the rate of O_2 uptake diminished rapidly from the time

the reaction commenced (Fig. 1). The oxidation stopped before two equivalents of O_2 had been taken up, unless a relatively large quantity of enzyme was initially present. Maximum O_2 uptake with the lowest enzyme concentration represented incomplete oxidation of catechin; and a second addition of enzyme resulted in an immediate acceleration of O_2 uptake (Fig. 1). Owing to the rapid inactivation of the enzyme during the reaction, it was difficult to obtain reliable measurements for the initial rate of oxidation, particularly if purified enzyme was used, and this experience is in agreement with the results obtained by earlier workers on the oxidation of catechol by mushroom polyphenoloxidase. To overcome this difficulty, Graubard & Nelson (1935) used the O_2 uptake at the end of 1 hr. as a measure of polyphenoloxidase activity, and the activity of the enzyme thus measured agreed with its activity similarly measured with catechol as substrate. Alternatively, by measuring O_2 uptake at 1 min.

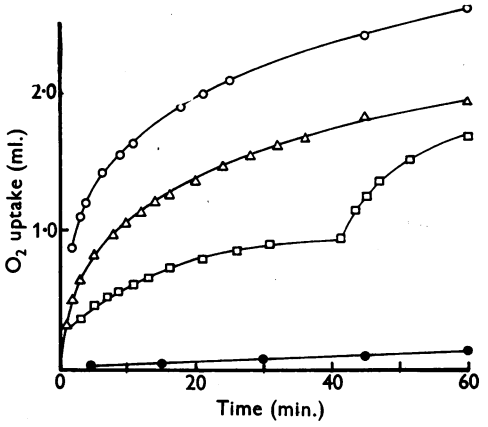

Fig. 1. Progress curves for the oxidation of catechin at 20° by mushroom polyphenoloxidase. In addition to 20 ml. of 0·07 M-phosphate buffer, pH 8, and 0·2 m-mole of catechin (●) the reaction flask contained 16 (○), 8 (△) and two separate additions of 4×10^{-3} E.U. (□). A compensatory flask contained phosphate buffer.

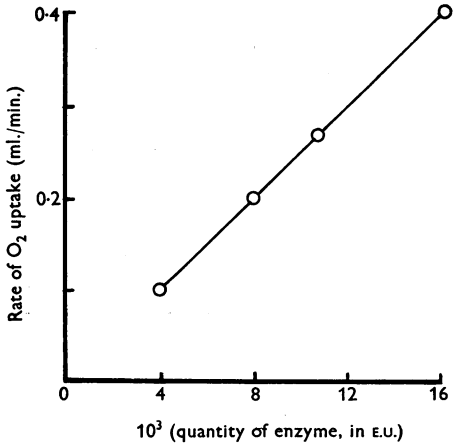

Fig. 2. Relationship between rate of O_2 uptake and enzyme concentration (mushroom enzyme).

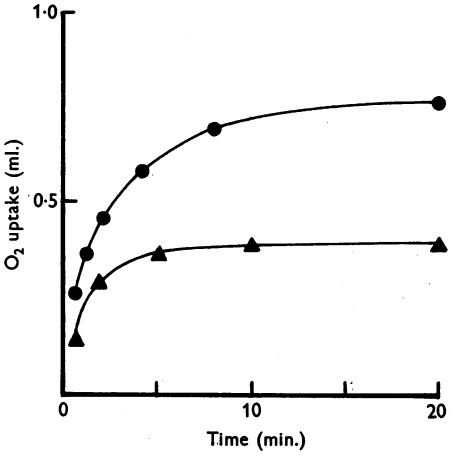

Fig. 3. Progress curves for the oxidation of catechin at 20° by potato polyphenoloxidase. Reaction flask contained 20 ml. of 0·07 M-phosphate buffer, pH 8, 0·2 m-mole of catechin and 8 (●) or 4×10^{-3} E.U. (▲). A compensatory flask contained phosphate buffer.

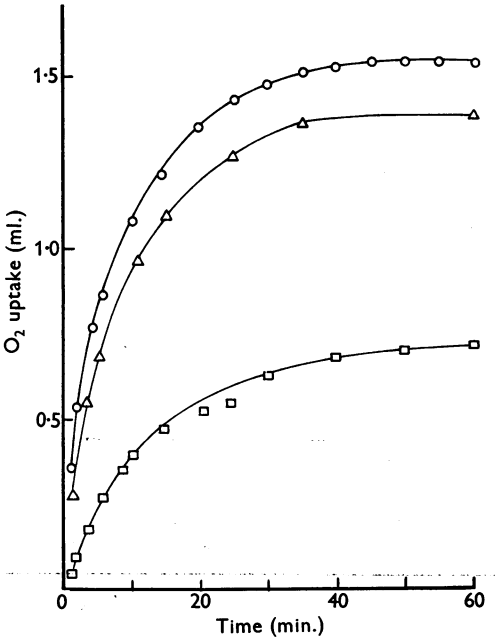

Fig. 4. Progress curves for the oxidation of catechin at 35° by tobacco polyphenoloxidase. Reaction flask contained 20 ml. of 0·07 M-phosphate buffer, pH 6, 0·2 m-mole of catechin and 2 (○), 0·7 (△) and $0·3 \times 10^{-3}$ E.U. (□). A compensatory flask contained phosphate buffer.

intervals for the first 2 or 3 min. (Keilin & Mann, 1938) and plotting the initial rate of oxidation observed against enzyme concentration, the usual linear relationship resulted (Fig. 2). The oxidation of catechin by potato polyphenoloxidase under identical temperature and pH conditions was also studied (Fig. 3), but the rate of enzyme inactivation was higher. The progress curves obtained for oxidation by tobacco polyphenoloxidase (Fig. 4) were similar to those for oxidation by mushroom enzyme, and O_2 uptake ceased before oxidation was complete unless a large amount of enzyme was present. Under the different optimum conditions of temperature (35°) and pH (6) for tobacco polyphenoloxidase (Reid, 1956), O_2 uptake at the end of 1 hr. was greater, at comparable levels of activity, for this enzyme than for the other enzymes studied.

The autoxidation and polyphenoloxidase oxidation of catechin gave polymers which had the same elementary analysis, remained at the origin in two-dimensional paper chromatography, were retained by hide-powder, and were precipitated by gelatin or on refluxing with a formalin–HCl mixture. The absorption spectra exhibited bands at 270 and 310 mμ and a shoulder at 500 mμ, but the shoulder at 500 mμ was slightly less intense with the autoxidation polymer (Fig. 5). The intensity of this shoulder decreases during the course of autoxidation, and this may be due to slight oxidative degradation by hydrogen peroxide, which is not formed during enzymic oxidation (cf. Beer, Broadhurst & Robertson, 1954). Figs. 5–7 were drawn from measurements made at 5 mμ intervals. Oxidation of catechin by hydrogen peroxide and horse-radish peroxidase gave a different polymer, which exhibited a single absorption band at 350 mμ.

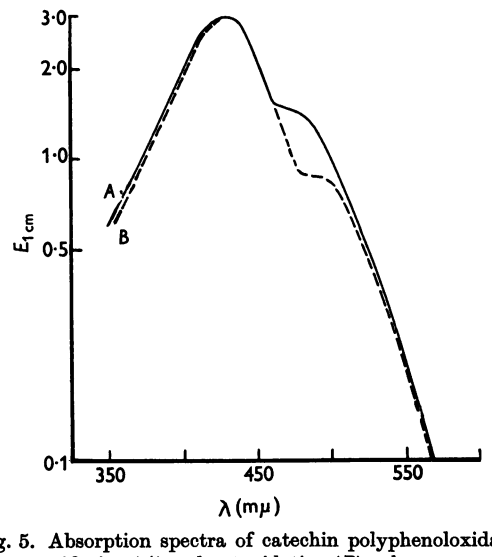

Fig. 5. Absorption spectra of catechin polyphenoloxidase oxidation (*A*) and autoxidation (*B*) polymers.

Percolation of *Acacia catechu* heartwood and freshly harvested *Uncaria gambir* leaves with water gave extracts, the residues of which contained 40 % of tamin; precautions were taken to prevent autoxidation during extraction. The residual aqueous solutions were examined after exhaustive solvent extraction, and found to contain approximately 50 % of the total tannin. Two-dimensional paper chromatograms in both cases revealed a single phenolic component at the origin. Electrodialysis of the residual solutions, in a cell fitted with ion-exchange membranes, precipitated the tannins, which were separated in the centrifuge after elution. Controlled drying furnished analytical specimens with elementary analyses (Table 1) similar to those for the polymer of catechin oxidation, of empirical formula $C_{15}H_{10}O_6,3H_2O$. The i.r. spectra provided evidence for hydration, for even after protracted drying at 70° *in vacuo* a strong band persisted at 1627–1635 cm.$^{-1}$. The absorption spectra of these phlobatannins at pH 8 (Fig. 6) were similar to those of the polymers of catechin oxidation, exhibiting maxima at 270, 410 and 500 mμ. These substances are retained by hide-powder, they do not give the ferric and vanillin reactions and they are precipitated by gelatin or on boiling with a formalin–HCl mixture.

The aerobic oxidation of an equimolecular mixture of *homo*catechol and phloroglucinol by mushroom polyphenoloxidase gave a polymer which showed absorption at 460 mμ (Fig. 7), in contrast to the single absorption band at 250 mμ of catechol autoxidation polymer (Hathway & Seakins 1957*a*). *homo*Catechol and 5-methoxy-4-methylresorcinol (Robertson & Whalley, 1951) substrates afforded a polymer which showed a strong absorption maximum at 480 mμ.

A flavonoid fraction derived from *Acacia catechu* heartwood was found to contain in addition to quercetin (Perkin, 1897) two flavonols, chromatographically indistinguishable in two different

Table 1. *Phlobatannin analyses*

	Found (%)		
	C	H	Empirical formula
Acacia catechu tannin	54·2	5·5	—
Catechin autoxidation polymer	53·0	4·7	$C_{15}H_{10}O_6,3H_2O$
Uncaria gambir tannin	52·0	5·7	—

solvents (Bate-Smith, 1949) from fisetin and quercetagetin (Table 2). Authentic quercetagetin was isolated from *Tagetes erecta* flowers for the purpose of comparison.

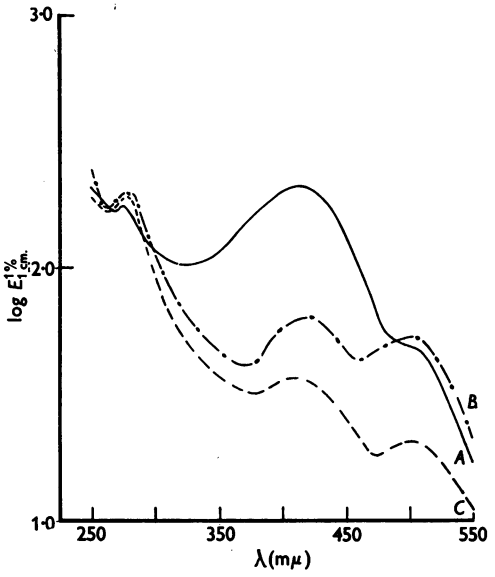

Fig. 6. Absorption spectra of catechin autoxidation polymer (*A*), and of tannins from *Acacia catechu* (*B*) and *Uncaria gambir* (*C*) in 0·07 M-phosphate buffer, pH 8.

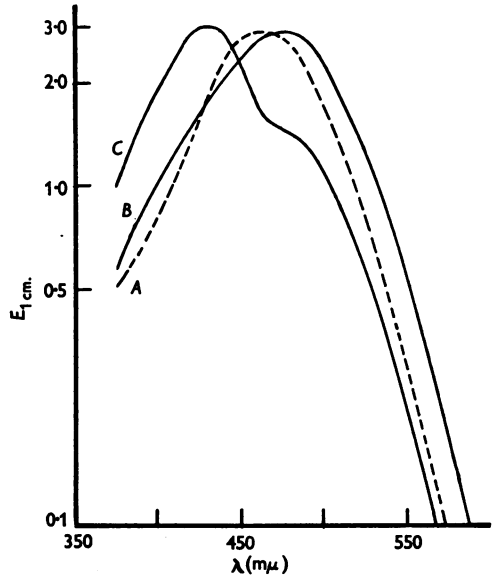

Fig. 7. Absorption spectra of the polymers resulting from aerobic oxidation of *homo*catechol and phloroglucinol (*A*), *homo*catechol and 5-methoxy-4-methylresorcinol (*B*), and catechin (*C*), by polyphenoloxidase.

DISCUSSION

A study of the course of aerobic oxidation of catechin by different plant polyphenoloxidases suggests that this substance functions as a substrate for these enzymes, which are widely distributed throughout plants. Both those polyphenoloxidases which are associated with the mitochondria, such as mushroom enzyme (Mason, 1955), and those which are not localized within the cell, such as tobacco enzyme (McClendon, 1953), have a common pattern of behaviour.

The products of polyphenoloxidase oxidation have been shown to be precisely similar to the polymer of catechin autoxidation which was formed by the quinone-polymerization mechanism. Additional evidence for the head-to-tail polymerization of catechin was also obtained from the aerobic oxidation of a mixture of *homo*catechol and 5-methoxy-4-methylresorcinol by polyphenoloxidase to a polymer which showed the characteristic absorption of the polymers of catechin oxidation. 4-Methyl-*o*-benzoquinone and 5-methoxy-4-methylresorcinol therefore undergo oxidative coupling. Aerobic oxidation of catechin by plant polyphenoloxidases proceeds by a quinone-polymerization mechanism and affords a product, the head-to-tail polymer units of which account for the profound difference in spectrum of the polymers from those of catechol polymers (Hathway & Seakins, 1957*a*). The fact that 4:5-dimethylcatechol does not undergo oxidative coupling with 5-methoxy-4-methyl-

Table 2. *Chromatographic behaviour of flavonols*

Solvent system *A*: *m*-cresol–acetic acid–water (50:2:48, by vol.; upper phase); solvent system *B*: butan-2-ol–acetic acid–water (14:1:5, by vol. v/v). Values given in parentheses were obtained with known compounds, the remainder with compounds obtained from *Acacia catechu*.

Compound	R_F *A*	R_F *B*
Fisetin	0·37 (0·38)	0·72 (0·75)
Quercetin	0·23 (0·23)	0·82 (0·83)
Robinetin	— (0·19)	— (0·61)
Quercetagetin	0·13 (0·13)	0·42 (0·44)
Myricetin	— (0·09)	— (0·57)

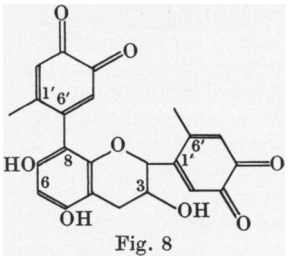

Fig. 8

resorcinol suggests that in these head-to-tail polymer units the 6′-position of one catechin-*o*-quinone residue is linked by a CC bond to the 6- or 8-position of another catechin-*o*-quinone residue. A partial type formula is shown in Fig. 8.

The polymer obtained by the enzymic oxidation of catechin has the analytical properties of a tannin (Gnamm, 1949; Schmidt, 1955). The polymer is precipitated from solution by gelatin or on refluxing with formalin–hydrochloric acid mixture, and it is retained by hide-powder (Grassmann *et al.* 1951). The failure of this polymer to give the vanillin reaction (Procter & Paessler, 1901) provides further evidence that polymerization involves the phloroglucinol residue of the monomer. The work of the Heidelberg School for 20 years has maintained (Freudenberg, 1956; Freudenberg & Maitland, 1934*a*, *b*; Freudenberg & Weinges, 1955) that phlobatannins from the barks of *Acacia mollissima*, birch, chestnut (*Castanea sativa*), eucalypts, mangrove, oak, spruce and willow and from the heartwood of *Acacia catechu* and quebracho trees are catechins, polymerized *post mortem* by plant acids. Two mechanisms have been considered. According to the first (Freudenberg & Maitland, 1934*a*, *b*), ring fission gives a secondary benzyl alcohol, and carbon atom $C_{(2)}$ then condenses either with carbon atom $C_{(6)}$ or with $C_{(8)}$ of another molecule to afford a bifunctional dimer, capable of further polymerization. More recently, Freudenberg (1956) has suggested that catechin may react as a pair of tautomeric diphenylpropenes which are involved in the first stage of a styrene polymerization (Freudenberg & Ahlhaus, 1956).

Phlobatannins have now been isolated in high yield from the tannin extractives of *Uncaria gambir* leaves and *Acacia catechu* heartwood, which are known to contain large quantities of catechin and its epimeride respectively. Since these substances have identical tannin properties, similar absorption spectra and elementary analyses to the polymer produced by polyphenoloxidase oxidation of catechin, a close structural relationship exists, and it is probable that these phlobatannins result from similar aerobic oxidation of catechin precursors. Formation of phlobatannins from catechin epimers by quinone polymerization recalls the similar formation of artificial melanins from 5:6-dihydroxyindoles (Beer *et al.* 1954; Bu'Lock & Harley-Mason, 1951), but it was not known whether the products resembled melanins of animal or plant origin (A. Robertson, personal communication). The fact that these new phlobatannins may be accounted for by catechin polymerization is in agreement with Freudenberg & Maitland's (1934*a*, *b*) suggestion, but no evidence has been found for the acid-catalysed reaction, which requires low pH (<2) and high temperature ($>50°$). The remaining phlobatannins from *Acacia catechu* and *Uncaria gambir* were shown by two-dimensional paper chromatography to contain mixtures of mobile substances. The absorption spectra of the crude mixtures were similar to those of the catechin oxidation polymers, but solubility in solvents and mobility in chromatographic-solvent systems implied a lower degree of polymerization.

Unlike the *Acacia catechu* and *Uncaria gambir* phlobatannins, a phlobatannin was isolated during the course of this work from a commercial extract derived from *Acacia mollissima* bark, which gave a vanillin reaction, a strong-violet ferric reaction, and a different absorption spectrum. It is therefore inadmissible to regard the phlobatannins at present as members of a closely related chemical family, but the presence of catechin derivatives in the cacao bean (Forsyth, 1952*a*) and tea leaf (Roberts, 1952), and of leucoanthocyanins in the bark of various eucalypts (Hillis, 1954, 1956) may indicate that the phlobatannins arise through quinone polymerization.

The present conclusion that enzymic formation of *Uncaria gambir* and *Acacia catechu* phlobatannins occurs in the detached leaves and heartwood respectively connects the formation of these compounds with the plant-browning reaction (Szent-Györgi & Vietorisz, 1931).

SUMMARY

1. Catechin was oxidized aerobically by mushroom, potato and tobacco polyphenoloxidases.

2. Catechin autoxidation and polyphenoloxidase oxidation polymers analyse as $C_{15}H_{10}O_6,3H_2O$, exhibit absorption bands at 270 and 410 mμ, and a shoulder at 500 mμ, and have identical analytical properties.

3. Aerobic oxidation of mixed synthetic substrates, such as *homo*catechol and 5-methoxy-4-methylresorcinol by polyphenoloxidase, gave a polymer showing the characteristic absorption of the polymers of catechin oxidation.

4. Aerobic oxidation of catechin by polyphenoloxidase therefore involves quinone polymerization.

5. Phlobatannins have been isolated in high yield from the extractives of harvested *Uncaria gambir* leaves and *Acacia catechu* heartwood, which have the same analytical properties as and similar elementary analyses and absorption spectra to the polymers of catechin oxidation.

6. The conclusion is drawn that these phlobatannins are formed by aerobic oxidation of catechin epimers by polyphenoloxidases in the detached leaves and heartwood respectively.

7. A flavonoid fraction from *A. catechu* contained three flavonols, chromatographically indistinguishable from fisetin, quercetagetin and quercetin.

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The Characteristics of Hexokinase from *Locusta migratoria* Muscle

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Hexokinases from different tissues vary in their properties and differ in particular in specificity towards their sugar substrates. Those enzymes which phosphorylate a number of sugars, including both glucose and fructose, have been described conveniently by Colowick (1951) as 'non-specific', whilst Medina & Sols (1956) suggest that the term ketokinase should be used for those which attack more than one ketose, but not aldoses, and that the name fructokinase be reserved for enzymes which act only with fructose. Non-specific hexokinases have been shown to be present in yeast (Berger, Slein, Colowick & Cori, 1946; Kunitz & McDonald, 1946), in higher plants (Saltman, 1953) and in a number of animal tissues (e.g. brain, Slein, Cori & Cori, 1950; retina, Hoare & Kerly, 1954; intestine, Sols, 1956). The specificity of the enzyme in heart muscle is similar to that of the enzyme in brain (Crane & Sols, 1955), but more than one enzyme may occur in mammalian skeletal muscle. Crane & Sols (1955) describe preparations of the non-specific enzyme from rat and dog muscle and Meyerhof (1927), in his early experiments on rabbit-muscle extracts, found that lactic acid was produced from glucose, mannose and fructose. Slein *et al.* (1950) showed that similar crude extracts could be fractionated to yield a partially purified enzyme which phosphorylated fructose but had little or no

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