300. *Autoxidation of Polyphenols. Part III.* Autoxidation in Neutral Aqueous Solution of Flavans related to Catechin.*

By D. E. HATHWAY and J. W. T. SEAKINS.

The formation of polymers and hydrogen peroxide during the autoxidation of catechin and of related 3′ : 4′-dihydroxyflavans has been studied by measurement of oxygen adsorption, and by elementary analyses, absorption spectra, and colour reactions of the dialysed polymers. The evidence obtained, together with that provided by spectroscopic study of intermediates produced by silver oxide oxidation, supports the theory that tannin formation from catechin occurs by polymerisation through quinones (Part II). Whereas polymerisation through quinones of catechin and 5 : 7 : 3′ : 4′-tetrahydroxyflavan involves the phloroglucinol residue, oxidative coupling of 5 : 7-di-*O*-methylcatechin and 3′ : 4′-dihydroxyflavan resembles that of catechol. The relevance of catechin autoxidation to the biogenesis of phlobatannins is considered.

IN Part II, a manometric study of catechin autoxidation, and the spectroscopic study of an intermediate obtained by silver oxide oxidation, supported the theory that tannin formation from catechin occurs by polymerisation through quinones.

Consideration of the data accumulated from autoxidation studies on simpler substances (Tables 1 and 2 and Figures 1 and 2) confirms that catechin autoxidation involves polymerisation of quinones, but that constitutional differences modify this process in the case of the flavans.

Manometry of the Polyhydroxyflavans.—Free hydroxyl groups in the 3′- and the 4′-positions (Table 1) cause an accumulation of hydrogen peroxide which is associated with quinone formation (cf. Part II). 5 : 7-Dihydroxy- and 5 : 7-dihydroxy-3′ : 4′-dimethoxy-flavans lack vicinal hydroxyl groups and can be recovered almost quantitatively after several days' autoxidation. Adsorption of oxygen in excess of the theoretical 1 mol. may be attributed to (1) manometric difficulties associated with protracted experiments (cf. the rapid oxidation of 5 : 6-dihydroxyindoles to melanins [1]), (2) two oxidative couplings per mole of *o*-quinone, or (3) oxidative degradation of some of the *o*-quinonoid residues with incorporation of more oxygen in the polymer. The possibility of experimental error in the total adsorption readings is the most likely, for multiple coupling (2) would lead to polymers exhibiting general light absorption like melanin, but some oxidative degradation of *o*-quinones by peroxide may take place even under the mild conditions of autoxidation.[1] Since the maximum rates of oxygen adsorption were determined at an early stage in these experiments, their accuracy is not subject to the same limitations. The similar manometric results for catechin and 5 : 7 : 3′ : 4′-tetrahydroxyflavan autoxidation are consistent with a common mechanism. In the present study the suggestion [2] that in acid solution catechin may polymerise as a reactive flaven has been tested by comparison of the behaviour of catechin and 5 : 7 : 3′ : 4′-tetrahydroxyflavan. Autoxidation of the bifunctional catechin molecule is slow, since oxidation to quinone is inhibited by phenols,[3] and the faster rate of autoxidation of the 5 : 7-di-*O*-methyl derivative agrees with this principle. Since the rate of autoxidation of 5 : 7 : 3′ : 4′-tetrahydroxyflavan is faster than that of catechin, the secondary alcoholic group in catechin exerts a steric factor [4] which lowers the rate of autoxidation, and the relative rates of autoxidation of 5 : 7-di-*O*-methylcatechin and 3′ : 4′-dihydroxyflavan are consistent with this view.

---

* Part I, *J.*, 1957, 519. The paper by Hathway and Seakins (*Nature*, 1955, **176**, 218) is considered to be Part II.

[1] Beer, Broadhurst, and Robertson, *J.*, 1954, 1947.

[2] Freudenberg and Weinges, *Annalen*, 1955, **590**, 140.

[3] James, Snell, and Weissberger, *J. Amer. Chem. Soc.*, 1938, **60**, 2084.

[4] Bu'Lock and Harley-Mason, *J.*, 1951, 709.

Polymer Analysis.—To obtain correspondence between the analyses of the dialysed oxidation products (Table 2) and those of true polymers, a calculated addition of 1·7—3·3 moles of water per mole of starting materials must be made, but a similar assumption was made for the melanins.[1] Evidence for hydration is found in the persistence of a strong spectral band at 1627—1635 cm.$^{-1}$ after protracted drying of the polymer at 70° *in vacuo.* Hergert and Kurth [5] using similar evidence found that (+)-catechin retained water after being dried at 115° *in vacuo* for several weeks. Unfortunately retention of water by the polymers prevents an examination of the fundamental carbon–oxygen stretching vibration in the 1650—1720 cm.$^{-1}$ region. Since these oxidation polymers are hydrated and difficult to analyse, no acceptable conclusion may be drawn from the analyses regarding the secondary oxidation of some of the *o*-quinone units, or the extent of multiple coupling of *o*-quinone.

FIG. 1. *Absorption spectra of polymers from* (*A*) *catechol,* (*B*) *5 : 7-di-O-methylcatechin, and* (*C*) *3′ : 4′-dihydroxyflavan.*

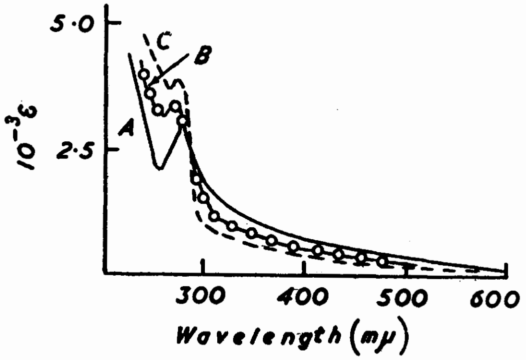

FIG. 2. *Absorption spectra of polymers from* (I) *catechin and* (II) *5 : 7 : 3′ : 4′-tetrahydroxyflavan.*

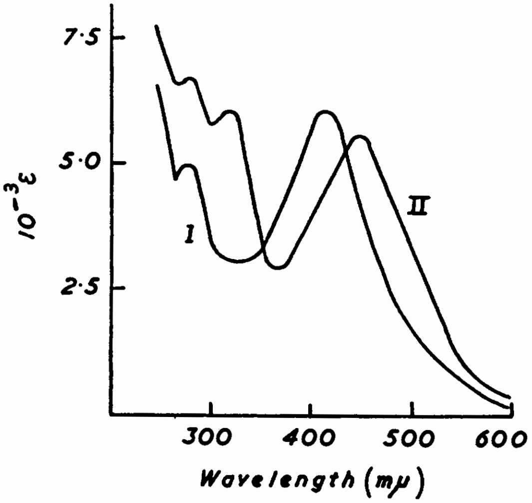

TABLE 1. *Oxygen adsorption by polyhydroxyflavans at* 35°.

	Oxygen uptake [a]	Residual peroxide [a]	Oxygen balance [a]	Maximum rate [b]	Reaction period [c]
Catechin	1·59	0·38	1·40	$5{\cdot}6 \times 10^{-4}$	2
5 : 7-Di-*O*-methylcatechin	2·00	0·39	1·80	$8{\cdot}4 \times 10^{-3}$	1·25
5 : 7-Dihydroxyflavan	—	0	—	$1{\cdot}9 \times 10^{-4}$	>7
5 : 7-Dihydroxy-3′ : 4′-dimethoxyflavan	—	0	—	$5{\cdot}3 \times 10^{-4}$	>7
3′ : 4′-Dihydroxyflavan	1·90	0·37	1·70	$1{\cdot}2 \times 10^{-2}$	1·25
5 : 7 : 3′ : 4′-Tetrahydroxyflavan	2·00	0·80	1·60	$3{\cdot}0 \times 10^{-3}$	1·25

The corresponding autoxidation of catechol, maximum rate $5{\cdot}6 \times 10^{-3}$ mole/min., was complete in 1 day.

[a] moles. [b] mole/min. [c] days.

TABLE 2. *Polymer analyses.*

	Found, %		Calc., %		
Polymers from :	C	H	C	H	Empirical formula
Catechin	52·6	4·6	53·0	4·7	$C_{15}H_{10}O_6,3H_2O$
5 : 7-Di-*O*-methylcatechin	57·7	5·5	57·7	5·5	$C_{17}H_{15}O_6,2{\cdot}2H_2O$
3′ : 4′-Dihydroxyflavan	67·1	5·4	67·1	5·4	$C_{15}H_{11}O_3,1{\cdot}7H_2O$
5 : 7 : 3′ : 4′-Tetrahydroxyflavan	54·5	5·1	54·6	5·1	$C_{15}H_{10}O_5,3{\cdot}3H_2O$

Polymer Properties.—Since the acid-catalysed polymer from catechin gives a vanillin reaction (Part II), the application of this reaction to the present polymers is justified.

---

[5] Hergert and Kurth, *J. Org. Chem.*, 1953, **18**, 521.

Unlike the corresponding monomers, the polymers from catechin and from 5 : 7 : 3′ : 4′-tetrahydroxyflavan do not react with vanillin reagent, whereas that from 5 : 7-di-*O*-methylcatechin gives a strongly positive reaction.

Absorption Spectra.—The absorption spectra of the polymers are of two classes. The polymers from 3′ : 4′-dihydroxyflavan and 5 : 7-di-*O*-methylcatechin have spectra almost identical with that of the polymer from catechol (Fig. 1), whereas the polymers from catechin and 5 : 7 : 3′ : 4′-tetrahydroxyflavan have spectra compatible with that expected for a polymer with a repeated quinone unit (Fig. 2). Compounds of the first class exhibit phenolic absorption at 280 mμ, and general absorption at 300—600 mμ. The similarity of the spectra of the polymers from 5 : 7-di-*O*-methylcatechin and catechol is consistent with a common mechanism of autoxidation. Methoxyl groups in the 5- and the 7-position of 5 : 7-di-*O*-methylcatechin therefore inhibit the head-to-tail polymerisation of the quinones. Substances of the second class exhibit a well-defined maximum at 410—430 mμ, and two maxima at shorter wavelengths.

Discussion.—Since polymerisation through quinones of catechol and 3′ : 4′-dihydroxyflavan involves catechol residues, the similarity of the spectra of the polymers obtained with that of the polymer from 5 : 7-di-*O*-methylcatechin is consistent with a common autoxidation mechanism. The possibility of some degree of head-to-tail polymerisation involving the phloroglucinol residues cannot be precluded from a consideration of the autoxidations of catechin and 5 : 7 : 3′ : 4′-tetrahydroxyflavan, however, and it is now suggested that head-to-tail polymer units of these substances may account for the profound difference in spectrum of the polymers from those of the catechol polymers.

For two decades the Heidelberg school has maintained[6,7] that the 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, polymerised *post-mortem* under the influence of the plant acids. Two mechanisms have been considered. According to the first,[6] ring fission gives a secondary benzyl alcohol, and carbon atom 2 then condenses with carbon atom 6 or 8 of another molecule to afford a bifunctional dimer, capable of further polymerisation. More recently, Freudenberg[8] has suggested that catechin may react as a pair of tautomeric diphenylpropenes which are involved in the first stage of a styrene polymerisation.[9] On the assumption that phlobatannins are indeed formed by polymerisation of catechin, then the enzymic autoxidation of catechin by polymerisation through quinones is a more attractive hypothesis, especially since the acid-catalysed reaction suggested by Freudenberg requires low pH (<2) and high temperature ($>50°$).

Polyhydroxyflavans.—The Simonis synthesis of flavanones from phloroglucinol and cinnamoyl chloride derivatives has been extensively studied,[10] and despite the customary low yields this reaction has been used for the preparation of eriodictyol and 5 : 7-dihydroxy-3′ : 4′-dimethoxyflavanone. Improved yields were obtained, however, by the use of ether instead of nitrobenzene as reaction medium. Clemmensen reduction at room temperature[11] of the flavanones gave the 5 : 7 : 3′ : 4′-tetrahydroxy- and 5 : 7-dihydroxy-3′ : 4′-dimethoxy-flavans. Partial methylation of (+)-catechin by means of methyl sulphate and aqueous alkali in the presence of excess of sodium metaborate[12] gave 5 : 7-di-*O*-methyl-(+)-catechin. 3′ : 4′-Dihydroxyflavan was prepared from 3 : 4 : 2′-trihydroxychalcone by catalytic hydrogenation, followed by benzoylation, and lithium aluminium

[6] Freudenberg and Maitland, *Annalen*, 1934, **510**, 193; *Collegium*, 1934, **776**, 656.

[7] Mayer and Bauni, *Das Leder*, 1956, **7**, 35; Schmidt and Mayer, *Angew. Chem.*, 1956, **68**, 103.

[8] Freudenberg, *Sci. Proc. Roy. Dublin Soc.*, 1956, **27**, 153.

[9] Freudenberg and Ahlhaus, *Monatsh.*, 1956, **87**, 1.

[10] (*a*) Shinoda and Sato, *J. Pharm. Soc. Japan*, 1928, **48**, No. 558, 109; 1928, **48**, No. 560, 117; (*b*) 1929, **49**, No. 563, 5; (*c*) 1931, **51**, 78; Shinoda and Kamagoye, 1928, **48**, No. 560, 119; Shinoda, Kamagoye, and Sato, 1931, **51**, 23; Shinoda, Sato, and Kamagoye, 1929, **49**, No. 571, 123; Fujise and Mitui, *Bull. Chem. Soc. Japan*, 1934, **9**, 24; Huzise and Tatsita, *Ber.*, 1941, **74**, 275.

[11] Robertson, Whalley, and Yates, *J.*, 1950, 3119.

[12] Anand, Gupta, Pankajamani, and Seshadri, *J. Sci. Ind. Res. India*, 1956, **15**, *B*, 263.

hydride reduction. 3 : 4 : 2′-Trihydroxychalcone was prepared by the condensation of 3 : 4-dihydroxyacetophenone [6] and salicylaldehyde in strongly alkaline solution. Methylation of 3′ : 4′-dihydroxyflavan gave 3′ : 4′-dimethoxyflavan, which was also synthesised from the available 2′-benzyloxy-αβ-dihydro-3 : 4-dimethoxychalcone.[13] The attempted preparation of 5 : 7 : 3′ : 4′-tetrahydroxy-6 : 8-dimethylflavan by Simonis synthesis from *CC*-dimethylphloroglucinol [14] and caffeyl chloride dibenzoate, followed by Clemmensen reduction, afforded crystals of the flavan which immediately resinified on exposure to air. Similar difficulty in the isolation of certain flavans has already been described.[15]

EXPERIMENTAL

General Procedure.—Solutions were concentrated in nitrogen under reduced pressure at $<35°$. Ferric chloride colours refer to reaction in ethanol.

Manometry.—Use was made of Haldane's constant-pressure respirometer,[16] 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. *n*-Butyl phthalate was used in the manometer and in the gas-burette. Both vessels, immersed in a thermostat at 35°, were shaken at a speed sufficient to maintain the reaction mixture as a foam. A small tube containing the flavan, and attached to a glass fibre, was placed upright in the reaction vessel, surrounded by phosphate buffer. In this way the system could be equilibrated before the reaction commenced by dislodging the tube and shaking the contents. A solution of 0·1 mmole of flavan in 10 ml. of phosphate buffer (pH 8), or phosphate buffer–methanol (1 : 1) (10 ml.) was used. The progress of the reaction was followed by direct reading of the volume of the system on the gas burette, the movable limb of which was first adjusted so as to bring the liquid in both arms of the constant-pressure manometer to the same level. When absorption of oxygen ceased, no flavan could be detected in the paper chromatogram of the reaction mixture. After acidification of the mixture, the polymer was adsorbed on a column (10 × 1·2 cm.) of alumina, prepared in 2N-sulphuric acid. Potassium iodide (1 g.) was added to the sulphuric acid eluate which was set aside (15 min.) in the dark, and the liberated iodine was estimated with 0·01N-sodium thiosulphate. In another experiment, the oxidising agent was shown to be hydrogen peroxide, as the distillate gave a positive reaction with titanic sulphate and was indistinguishable from distilled water between 300—700 mμ.

Spectrophotometric Experiments and Analytical Samples.—A solution of the flavan (1 mmole) in phosphate buffer (pH 8; 100 ml.) or its equivalent of phosphate buffer–methanol (1 : 1) was shaken with oxygen until no more was absorbed. Samples were then withdrawn for spectrophotometric examination of the polymer, the solution being diluted where required with more phosphate buffer. For the isolation of the pure polymer phosphate ion was removed by introducing the reaction mixture into the middle compartment of an electrodialysis cell, constructed from "Pyrex" pipe-line,[17] and fitted with a "Permaplex" A-10 anion-exchange membrane, and a "Permaplex" C-10 cation-exchange membrane. The cell was supplied with D.C. at 100 v until the resistance approached a limit (*ca.* 10 hr.); the solution was then free from phosphate ion (molybdenum-blue test). Removal of phosphate ion caused precipitation of the polymer which was removed in the centrifuge, and dried (3 days at 20°/0·01 mm., followed by 8 hr. at 70°/0·01 mm. over P_2O_5). All the polymers were ashless on combustion.

Vanillin reaction. A 10% (w/v) ethanolic solution of vanillin was mixed with 12N-hydrochloric acid (2 : 1 v/v) and the freshly prepared reagent was employed at room temperature.

5 : 7-*Dihydroxyflavan.*—This had m. p. 196° (Found : C, 74·2; H, 5·9. Calc. for $C_{15}H_{14}O_3$: C, 74·4; H, 5·8%) when prepared by the method of Robertson, Whalley, and Yates.[11]

5 : 7-*Di-O-methyl-*(+)-*catechin.*—Aqueous 4M-sodium metaborate (10 ml.), methyl sulphate (2 ml.), and 2·5N-sodium hydroxide (8 ml.) were shaken with an ethanolic solution of (+)-catechin (2·9 g.) for 15 min.; the reaction mixture was then poured into ice-water and extracted with ether. The acidified aqueous phase was extracted with ether (4 × 100 ml.), and the

---

[13] Freudenberg, *Annalen*, 1925, **441**, 179.

[14] Robertson and Whalley, *J.*, 1951, 3355.

[15] Robertson, Venkateswarlu, and Whalley, *J.*, 1954, 3137.

[16] Haldane, *J. Path. Bact.*, 1921, **23**, 443; Dixon, "Manometric Methods," University Press, Cambridge, 1934, p. 6.

[17] Lovering and Smith, *Chem. and Ind.*, 1946, **24**, 298.

ether phase was washed free from mineral acid and dried. Evaporation of the solvent ($MgSO_4$) left 5 : *7-di-O-methyl-(+)-catechin* (2·6 g.), forming needles (from aqueous ethanol), m. p. 218—219°, $[\alpha]_D^{20}$ −1·0 (*c*, 1·1 in acetone; *l*, 1) {(+)-catechin, $[\alpha]_D^{20}$ +12° (*c*, 1·7); 5 : 7 : 3′ : 4′-tetra-*O*-methyl-(+)-catechin $[\alpha]_D^{20}$ −2° (*c*, 1·0)} (Found: C, 64·0; H, 5·6; OMe, 19·1. $C_{17}H_{18}O_6$ requires C, 64·0; H, 5·6; OMe, 19·1%). 5 : 7-Di-*O*-methyl-(+)-catechin gave a green ferric reaction, and a positive vanillin reaction.

5 : 7-*Dihydroxy*-3′ : 4′-*dimethoxyflavan*.—5 : 7-Dihydroxy-3′ : 4′-dimethoxyflavanone, m. p. 202° (Found: C, 64·9; H, 5·2; OMe, 19·7. Calc. for $C_{17}H_{16}O_6$: C, 64·6; H, 5·1; OMe, 19·5%), was prepared in 25% yield by an improvement of Shinoda and Sato's [10b] method. Reduction of the flavanone by Robertson, Whalley, and Yates's method [11] gave 5 : 7-*dihydroxy*-3′ : 4′-*dimethoxyflavan*, nodules (from xylene), m. p. 260° (Found: C, 67·3; H, 5·9. $C_{17}H_{18}O_5$ requires C, 67·5; H, 5·9%), which gave a positive vanillin reaction but no ferric reaction.

5 : 7 : 3′ : 4′-*Tetrahydroxyflavan*.—Eriodictyol, m. p. 267° (Found: C, 62·0; H, 4·0. Calc. for $C_{15}H_{12}O_6$: C, 62·5; H, 4·2%), was prepared in 20% yield (cf. Shinoda and Sato [10b]) by the method used for its 3′ : 4′-dimethyl ether. Reduction of eriodictyol by the method used for the 3′ : 4′-dimethyl ether gave the *flavan* which formed felted needles, m. p. 185° (from water) (Found: C, 62·1; H, 5·4. $C_{15}H_{14}O_5,H_2O$ requires C, 61·8; H, 5·5%), which were dried (P_2O_5) at 70°/0·1 mm. for 3 hr., and gave a green ferric reaction and a vanillin reaction.

3′ : 4′-*Dihydroxyflavan* (with Dr. W. E. Elstow [18]).—A mixture of an ethanolic solution (60 ml.) of 3 : 4-dihydroxyacetophenone [6] (19 g.) and salicylaldehyde (16 g.) and 40% (w/v) potassium hydroxide solution (150 ml.) was kept under nitrogen for 1 week. 3 : 4 : 2′-*Trihydroxychalcone* formed brown needles (30 g.) (from aqueous ethanol), m. p. 189° (Found: C, 70·3; H, 4·8. $C_{15}H_{12}O_4$ requires C, 70·3; H, 4·7%). An ethanolic solution of the chalcone (25·6 g.) was hydrogenated at 20°/1 atm. in the presence of Adams's platinic oxide catalyst (200 mg.), until hydrogen absorption (Found: 2·35 l.; Calc.: 2·30 l.) was complete. Evaporation of the filtrate left crude αβ-dihydro-3 : 4 : 2′-trihydroxychalcone which was benzoylated by the Schotten–Baumann method. 3 : 4 : 2′-*Tribenzoyloxy*-αβ-*dihydrochalcone* formed needles (55 g.) (from ethanol), m. p. 113° (Found: C, 75·6; H, 4·7. $C_{36}H_{26}O_7$ requires C, 75·8; H, 4·6%). Lithium aluminium hydride reduction of the dihydrochalcone (5·7 g.) gave 3′ : 4′-*dihydroxyflavan* which formed needles (2·6 g.) (from light petroleum [b. p. 60—80°]), m. p. 132° (Found: C, 74·4; H, 5·9. $C_{15}H_{14}O_3$ requires C, 74·4; H, 5·8%). The flavan gave a green ferric reaction.

3′ : 4′-*Dimethoxyflavan*.—Treatment at 0° of an ethereal solution of 3′ : 4′-dihydroxyflavan (2·6 g.) with ethereal diazomethane (*ca.* 2·8 g.) gave 3′ : 4′-*dimethoxyflavan* as thick lamellæ (2·6 g.), m. p. 99—100° (Found: C, 75·9; H, 6·5. $C_{17}H_{18}O_3$ requires C, 75·7; H, 6·7%), undepressed by another specimen, m. p. 99—100°, prepared by lithium aluminium hydride reduction of the corresponding 2′-benzoyloxy-αβ-dihydro-3 : 4-dimethoxychalcone.[13]

The authors thank Dr. H. Phillips for his interest, and the Council of the British Leather Manufacturers' Research Association for permission to publish this paper. They are also indebted to Professor R. D. Haworth, F.R.S., for the infrared spectrum of the polymer from catechin.

British Leather Manufacturers' Research Association,
Milton Park, Egham, Surrey.

[*Received, October 8th*, 1956.]

---

[18] Elstow, Ph.D. Thesis, London University, 1954.