1169. *Polymerisation of Flavans. Part VII.*[1] *Oxidative Polymerisation of Catechin.*

By B. R. Brown and R. J. Whiteoak.

Laccase-catalysed oxidation at pH 5 or autoxidation at pH 6 of catechin in presence of sodium benzenesulphinate yields 6′-phenylsulphonylcatechin which has been characterised as its methyl, acetyl, and tosyl derivatives. In absence of sodium benzenesulphinate both oxidations yield polymers. This constitutes further experimental evidence for the suggestion of other workers that oxidation of catechin leads to a monomeric *o*-quinone, or to its semiquinone radical, which then initiates polymerisation.

Flavans are known to polymerise when they are treated with acids or when they are oxidised. The mechanism of the acid-catalysed polymerisation is well understood, as are the structures of the resulting polymers.[2–4] Oxidative polymerisation by air, both uncatalysed and catalysed by enzymes, has been investigated and a mechanism has been suggested;[5] however, the details are not as well substantiated as are those of the acid-catalysed polymerisation. Oxidative polymerisation of phenolic flavans to yield tannin-like substances is thought to play a significant role in several processes of biological importance, *e.g.*, the browning of fruits and vegetables[6] and the development of resistance in plants towards fungal and viral infections.[7] It therefore seemed desirable to investigate in more detail the process of oxidative polymerisation of catechin.

Hathway and Seakins[5] are responsible for the most significant recent advances in the field of oxidative polymerisation of flavans and they suggest a mechanism for the autoxidation of catechin in aqueous solution which involves oxidation of catechin (I) to an *ortho*-quinone (II) and reaction of this with another molecule of catechin, the process being repeated to yield polymers of type (III). Evidence that quinone intermediates are involved came from the observation that autoxidation was arrested by sodium hydrogen sulphite, from measurement of oxygen uptake and hydrogen peroxide production, from the ultraviolet spectrum of the polymeric product ($\lambda_{max.}$ 400 mμ at pH 6 and 420—430 mμ at pH 8),[5b] and from the production by oxidation of catechin with silver oxide of a dioxan solution ($\lambda_{max.}$ 370 mμ)

[1] Part VI, *J.*, 1962, 1658.

[2] K. Freudenberg *et al.*, *Annalen*, 1934, **510**, 193; 1954, **590**, 140; *Ber.*, 1957, **90**, 957; *Annalen*, 1958, **612**, 78; *Chem. and Ind.*, 1959, 486; *Tetrahedron Letters*, 1962, 1073.

[3] B. R. Brown *et al.*, *J.*, 1957, 3757; 1958, 4302; 1961, 3677; *Tetrahedron Letters*, 1963, 905.

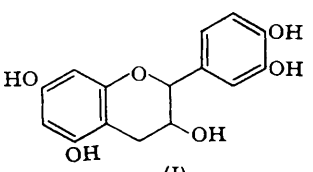
[4] W. Mayer *et al.*, *Chem. and Ind.*, 1959, 485; *Annalen*, 1961, **644**, 70; *Naturwiss.*, 1963, **50**, 152.

[5] (*a*) D. E. Hathway and J. W. T. Seakins, *Nature*, 1955, **176**, 218; (*b*) *J.*, 1957, 1562; (*c*) D. E. Hathway, *J.*, 1958, 520; (*d*) *Biochem. J.*, 1957, **67**, 445.

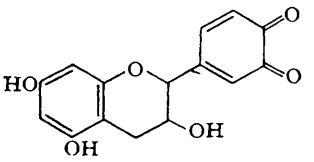
[6] *E.g.*, H. W. Siegelman, *Arch. Biochem. Biophys.*, 1955, **56**, 97.

[7] A. H. Williams, "Enzyme Inhibition by Phenolic Compounds," in "Enzyme Chemistry of Phenolic Compounds," ed. J. B. Pridham, Pergamon Press, Oxford, 1963, p. 87.

which reacted with catechin in phosphate buffer to yield polymeric material.[5a] The presence of a link at the 6-position of the polymer (III) was confirmed by isolation of *m*-hemipinic acid (2%) on vigorous oxidation of the reduced, methylated polymer.[5c] Whether linkage in ring A is at the 8-position as shown in the polymer structure (III), or at the 6-position, or at both, is not known. Hathway[5d] investigated the enzyme-catalysed oxidative polymerisation of catechin and suggested that its path was identical with that of the autoxidation.



(I)



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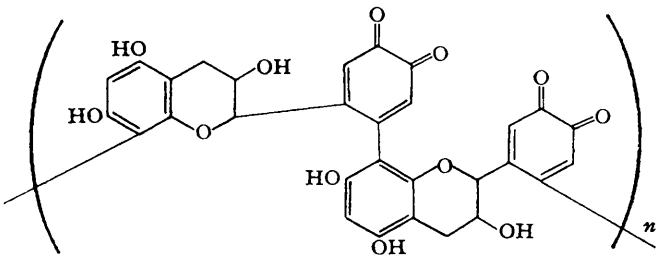


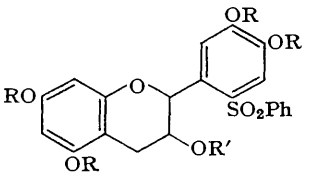
(III)

The observation[8] that addition of sodium benzenesulphinate to the laccase-catalysed oxidation of catechol or quinol yields the phenylsulphonyl derivatives of the related quinones, suggested to us that the phenyl sulphone corresponding to the quinone (II) might be isolated from an intermediate involved in the oxidative polymerisation of catechin. It should be mentioned that Lamb and Sreerangachar[9] attempted to trap the quinone (II) by addition of aniline to an enzyme-catalysed oxidation of catechin, but their success was severely limited by the impurity and small amount of anilino-derivative, which they were unable to characterise satisfactorily.

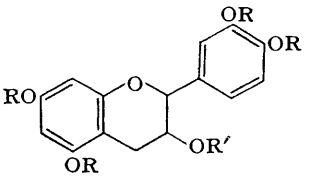
Laccase-catalysed Oxidation of Catechin.—Oxidation of (±)-catechin (obtained from Indian kath by recrystallisation from water) in presence of laccase from *Polyporus versicolor*[8] at pH 5 and 24° yielded polymer as a yellowish-brown powder which was immobile on a paper chromatogram run in 2% acetic acid. Oxidation of (±)-catechin in presence of laccase and sodium benzenesulphinate at pH 5 and 23° was followed by paper chromatography in 2% acetic acid, revealing the presence of compounds of R_F 0·56 (catechin has R_F 0·46), but complete absence of polymer. Isolation, methylation, and separation of the crude product yielded two crystalline solids: (i), in greatest amount, m. p. 201—203°, $[\alpha]_D$ 0° and (ii) m. p. 192—193°, $[\alpha]_D$ +81·8°. The first compound is formulated as (±)-tetra-*O*-methyl-6′-phenylsulphonylcatechin (IV; R = Me, R′ = H), corresponding to the quinone (II), on the following evidence. Elemental analysis of the compound, its 3-acetate (IV; R = Me, R′ = Ac), and 3-*p*-tosylate (IV; R = Me, R′ = Ts), and a molecular weight determination (by osmometry) on the 3-acetate were consistent with structure (IV; R = Me, R′ = H). The proton magnetic resonance spectra of the compounds in deuterochloroform provided evidence for the position of the phenylsulphonyl group.

[8] G. Benfield, Sheila M. Bocks, K. Bromley, and B. R. Brown, *Phytochemistry*, 1964, **3**, 79.
J. Lamb and H. B. Sreerangachar, *Biochem. J.*, 1940, **34**, 1472.

The aromatic protons of ring A are seen as a pair of doublets ($J = 2$–3 c./sec.) (see Table), the higher field doublet corresponding to the C-6 proton, as determined for flavans by Massicot and Marthe[10] and reported also by Waiss *et al.*[11] for the trimethylsilyl ethers of various



(IV)



(V)

TABLE

Proton magnetic resonance spectra: chemical shifts (τ units) and coupling constants (c./sec.) of the aromatic protons of catechin derivatives.

Compound	H-6	H-8	H-2′	H-5′	H-6′	$J_{6,8}$
(V; R = R′ = H)*	4·21(d)	4·02(d)	3·19(s)		(1 proton)	2·4
			3·26(s)		(2 protons)	
(IV; R = R′ = H)*	4·45(d)	4·06(d)	3·05(s)	2·49(s)	—	2·4
(V; R = Me, R′ = H)	3·83(s)		3·00(s)		(2 protons)	0
			3·02(s)		(1 proton)	
(IV; R = Me, R′ = H)	4·01(d)	3·84(d)	2·78(s)	2·28(s)	—	2·1
(V; R = Me, R′ = Ac)	3·87(d)	3·78(d)	3·06 (slight splitting)			2·4
(IV; R = Me, R′ = Ac)	4·08(d)	3·86(d)	2·88(s)	2·23(s)	—	3·0
(IV; R = Me, R′ = Ts)	4·05(d)	3·85(d)	3·28(s)	2·36(s)	—	3·0
(V; R = R′ = Ac)	3·36(d)	3·29(d)		2·74(s)		2·1
(IV; R = R′ = Ac)	3·60(d)	3·37(d)	2·59(s)	1·90(s)	—	3·0

* Measured in dimethyl sulphoxide solution. d = doublet; s = singlet.

flavanoids, including catechin, and by Batterham and Highet.[12] For comparison, the Table shows the values for the catechin derivatives without the sulphone substituent. The values obtained for the C-6 and C-8 protons of catechin itself (τ 4·21 and 4·02, respectively, in dimethyl sulphoxide) are in good agreement with those recently reported by Batterham and Highet for (+)-catechin (τ 4·27 and 4·07, respectively, in deuterated dimethyl sulphoxide). The C-6 and C-8 protons of tetramethylcatechin are evidently equivalent, appearing as a singlet at τ 3·83. Whereas the ring B protons of the catechin derivatives are almost equivalent, giving signals with very little spin-spin splitting, when the phenylsulphonyl substituent is present, the remaining ring B protons appear as two singlets with widely differing chemical shifts. As no spin-spin splitting is observed, this pattern provides good evidence for the protons being situated *para* to each other, thus indicating that the phenylsulphonyl group has entered the C-6′ position as expected. The shift to lower field of the C-5′ proton is expected because of the shielding effect of this substituent. Integration of the spectra provided additional evidence for the monomeric nature of the compounds.

The second compound was shown to be structurally isomeric with (±)-tetra-*O*-methyl-6′-phenylsulphonylcatechin and admixture of it with (−)-tetra-*O*-methyl-6′-phenylsulphonylcatechin (see below) to yield the (±)-phenyl sulphone showed it to be (+)-tetra-*O*-methyl-6′-phenylsulphonylcatechin [2R,3S-configuration as in (−)-catechin]. Thus the catechin used in the oxidation was very probably (±)-catechin containing a small amount of (−)-catechin in excess, a conclusion consistent with its slight negative rotation and with the wide m. p. and slight negative rotation of its tetramethyl ether. Kath is a form of cutch obtained from *Acacia catechu*[13] which contains (+)-catechin and (−)-epicatechin. The process of

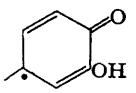
[10] J. Massicot and J-P. Marthe, *Bull. Soc. chim. France*, 1962, 1962.
[11] A. C. Waiss, R. E. Lundin, and D. J. Stern, *Tetrahedron Letters*, 1964, 513.
[12] T. J. Batterham and R. J. Highet, *Austral. J. Chem.*, 1964, **17**, 428.
[13] F. N. Howes, "Vegetable Tannin Materials," Butterworths, London, 1953, p. 154.

extraction which involves prolonged evaporation of aqueous solutions at the b. p. probably causes racemisation and epimerisation in which the equilibria are known[14] to lie very much on the side of (+)-catechin and (−)-catechin. A slight excess of (−)-epicatechin in the original extract would account for the excess of (−)-catechin in the final product.

With the aid of a seed of crystalline material obtained from the autoxidation (see below), (±)-6′-phenylsulphonylcatechin (IV; R = R′ = H) was isolated from the crude reaction product of the laccase-catalysed oxidation of (±)-catechin and acetylation of the crude product yielded (±)-penta-acetyl-6′-phenylsulphonylcatechin (IV; R = R′ = Ac).

The laccase-catalysed oxidation of (+)-catechin was analogous to that of (±)-catechin and in presence of sodium benzenesulphinate yielded, after methylation, the (−)-tetramethyl-6′-phenylsulphone [2S,3R-configuration as in (+)-catechin], m. p. 190—192°, $[\alpha]_D$ −81·2°.

Autoxidation of Catechin.—The products from autoxidation of (±)-catechin in presence of sodium benzenesulphinate have been shown to be pH-dependent: at pH 8 only red polymeric material was obtained and reaction of the catechin was complete in 3·5 days; at pH 7 complete reaction took 9 days and the product was mainly polymer with a little (±)-6′-phenylsulphonylcatechin which was isolated as its tetramethyl ether; at pH 6 reaction was not complete even after 14 days and the product yielded (±)-6′-phenylsulphonylcatechin which was isolated and identified and, after methylation, (±)-tetramethylcatechin and (±)-tetramethyl-6′-phenylsulphonylcatechin. A small amount of polymer was detected in the oxidation at pH 6 by paper chromatography. This form of pH-dependence is that expected for a process in which a nucleophile reacts with an intermediate quinone (II) or with a related precursor, *e.g.*, the radical (VI). Competition for the intermediate can occur between the benzenesulphinate anion and catechin and since the efficiency of catechin as a



(VI)

nucleophile is increased by conversion into its anion, increase of pH will result in production of more polymer than phenylsulphonylcatechin. Autoxidation at pH 6 in presence of sodium benzenesulphinate yields phenylsulphonylcatechin with little polymer; enzymic oxidation at pH 5 yields phenylsulphonylcatechin with no detectable polymer.

Autoxidation of Catechol.—At pH 8 in presence of sodium benzenesulphinate catechol yields 4-phenylsulphonylcatechol and polymeric material; enzymic oxidation at pH 5 yields only 4-phenylsulphonylcatechol.[8]

Experimental

Infrared spectra were recorded on a Perkin-Elmer 237 spectrometer, ultraviolet spectra on a Unicam SP800 instrument, and proton magnetic resonance spectra were measured at 60 mc./sec. on a Perkin-Elmer spectrometer, with tetramethylsilane as an internal reference. Alumina refers to Type H of P. Spence & Sons Ltd., deactivated with 10% (v/w) of 10% aqueous acetic acid. Paper chromatography refers to Whatman No. 4 paper, run in 2% acetic acid and sprayed with ferric chloride–potassium ferricyanide solution.

The catechin used for most of the experimental work was crude kath, from *Acacia catechu*, recrystallised from methanol. In this form it was brown and an optical rotation could not be determined, but after treatment with charcoal and further crystallisation from aqueous ethanol, an almost colourless sample was obtained, $[\alpha]_D^{20}$ −1·6° (*c* 1·0 in 1:1 acetone–water). This rotation showed that the sample was (±)-catechin containing a small excess of (−)-catechin. The absorption spectra of both recrystallised samples in 0·1M-phosphate buffer (pH 8) showed an absorption maximum at 277·5 mμ with no maxima at higher wavelengths. Methylation with dimethyl sulphate and potassium carbonate in acetone afforded tetra-*O*-methylcatechin, $[\alpha]_D^{20}$ +1·9°

[14] K. Freudenberg and L. Purrman, *Annalen*, 1924, **437**, 274.

(*c* 1·9 in $CHCl_3$), m. p. 136—140° after repeated recrystallisation from methanol (lit. 142°). The optical rotation and low m. p. were consistent with the presence of a slight excess of (−)-catechin in the starting material.

The solution of laccase was obtained from *Polyporus versicolor* as previously described.[8]

Laccase-catalysed Oxidation of (±)-*Catechin.*—Enzyme solution (15 ml.) and a solution of (±)-catechin (1·0 g.) in 0·01M-acetate buffer of pH 5 (750 ml.) was kept at 24° with occasional swirling. A yellowish-brown solid began to separate after 1 day, and after 4 days a further quantity (15 ml.) of enzyme solution was added. After 6 days 2N-hydrochloric acid (30 ml.) was added. A sample (2 ml.) was withdrawn and diluted with methanol (2 ml.) to give a clear brown solution; $\lambda_{max.}$ 285 (shoulder) and 380 mμ. A portion of this solution was evaporated to dryness *in vacuo* below 50° and was dissolved in phosphate buffer of pH 8 to give a red-brown solution; $\lambda_{max.}$ 275 (shoulder), 430, and 490 (shoulder) mμ. The polymer (0·25 g.), which was precipitated after the addition of the mineral acid, was collected by centrifugation.

Laccase-catalysed Oxidation of (±)-*Catechin in Presence of Benzenesulphinic Acid.*—A solution of (±)-catechin (2·0 g.) and sodium benzenesulphinate (2·0 g.) in 0·01M-acetate buffer of pH 5 (1·7 l.) and enzyme solution (20 ml.) were stirred at 23° for 4 days. The progress of the reaction was followed by paper chromatography. The catechin spot (R_F 0·46) gradually disappeared as a new spot of R_F 0·56 appeared. The solution developed an orange colour. After 5 days, when all the catechin had reacted, the reaction was terminated by the addition of 2N-hydrochloric acid (60 ml.), and the solution was extracted with ethyl acetate (4 × 200 ml.). The organic extract was washed with water (200 ml.), then with brine (100 ml.), dried, and evaporated below 50°, to give a pale brown residue (2·91 g., 98%). Crystallisation of an aqueous methanolic solution of this residue was induced with difficulty by the addition of a seed of the crystalline (±)-6′-*phenylsulphonylcatechin* (IV; R = R′ = H), m. p. 164–168°, obtained from the autoxidation reaction (see later) (Found: C, 56·5; H, 4·55; S, 6·85. $C_{21}H_{18}O_8S,H_2O$ requires C, 56·25; H, 4·5; S, 7·15%); $\nu_{max.}$ (in Nujol) 1300 and 1140 cm.$^{-1}$ (sulphone S=O stretching). The sulphone group was also detected by spraying a chromatogram with a 20% solution of potassium iodide in 2N-hydrochloric acid,[15] when a red-brown spot slowly developed on a yellow background. As a check that the *ortho*-dihydroxy-groups were still present, the ultraviolet spectrum in ethanol containing boric acid and sodium acetate was compared with that in ethanol alone,[16] $\lambda_{max.}$ (in EtOH) 265 and 292 mμ; $\lambda_{max.}$ (in EtOH–H_3BO_3–NaOAc) 292 and 301 mμ [*cf.* catechin, $\lambda_{max.}$ (in EtOH) 282 mμ; $\lambda_{max.}$ (in EtOH–H_3BO_3–NaOAc) 289 mμ].

Since purification of the phenolic product was difficult, it was methylated as the amorphous form. Potassium carbonate (3·0 g.) and dimethyl sulphate (3·0 ml.) were added to a stirred solution of the phenolic sulphone (2·9 g.) in anhydrous AnalaR acetone (30 ml.), and the mixture was heated under reflux for 2 hr. More dimethyl sulphate (3·0 ml.) was added and the reaction was continued for a further 2·5 hr. Concentrated ammonium hydroxide (9 ml.) was added dropwise to the solution and most of the acetone was removed by evaporation. Water (30 ml.) was added to the cold mixture which was then extracted with chloroform (3 × 60 ml.). The extract was washed with water (50 ml.), then with brine (50 ml.), dried, and evaporated to yield an orange-brown gum (3·64 g.). Thin-layer chromatography (Merck Kieselgel type H, run in ethyl acetate, sprayed with aqueous toluene-*p*-sulphonic acid) showed predominantly one spot, of R_F 0·63, with several less intense, more diffuse spots of slightly lower R_F. The crude product was dissolved in benzene (30 ml.) and chromatographed on alumina (180 g.). Elution with 10% benzene in ether afforded two crystalline compounds, which could be separated by fractional crystallisation from chloroform–ether. The more abundant, which crystallised first, was recrystallised from methanol–ether to give (±)-*tetra*-O-*methyl*-6′-*phenylsulphonylcatechin* (IV; R = Me, R′ = H) (1·09 g.) as colourless prisms, m. p. 201—203°, containing methanol of crystallisation which could not be completely removed by prolonged drying at 100° *in vacuo*, $[\alpha]_D^{24}$ 0° (*c* 1·0 in $CHCl_3$) (Found: C, 60·7; H, 5·5; S, 6·55. $C_{25}H_{26}O_8S,\frac{1}{2}CH_3{\cdot}OH$ requires C, 60·95; H, 5·6; S, 6·4%); $\nu_{max.}$ (in Nujol) 3550sh (OH), 1310 and 1150 cm.$^{-1}$ (sulphone); $\nu_{max.}$ (in $CHCl_3$) 3500 cm.$^{-1}$ (broad, OH).

(±)-3-O-*Acetyltetra*-O-*methyl*-6′-*phenylsulphonylcatechin.*—Treatment of the sulphone with acetic anhydride in pyridine on a water-bath for 1·5 hr., removal of the solvent, and filtration of a benzene solution of the residue through a short column of alumina yielded the (±)-3-O-*acetyl-derivative* (IV; R = Me, R′ = Ac), which separated from ethyl acetate as rods, m. p. 214—218° [Found: C, 61·3; H, 5·2; S, 6·65%; *M* (osmometry in benzene), 513. $C_{27}H_{28}O_9S$ requires C, 61·35;

[15] C. de Marco, *Nature*, 1963, **198**, 683.
[16] L. Jurd, *Arch. Biochem. Biophys.*, 1956, **63**, 376.

H, 5·35; S, 6·05%; *M*, 528]; $\nu_{max.}$ (in Nujol) 1745 (ester C=O), 1310 (sulphone), 1220 (ester C–O), and 1150 cm.$^{-1}$ (sulphone).

(±)-*Tetra*-O-*methyl*-6′-*phenylsulphonyl*-3-O-*tosylcatechin*.—The sulphone was warmed on a water-bath with an excess of toluene-*p*-sulphonyl chloride in pyridine for 5 hr. The solution was diluted with chloroform, washed successively with N-hydrochloric acid, water, and brine, dried, and evaporated. Crystallisation of the residue from methanol afforded the (±)-3-O-*tosyl derivative* (IV; R = Me, R′ = Ts) as glistening plates, m. p. 190—195° (turning red at the m. p.) (Found: C, 59·7; H, 5·0; S, 9·1. $C_{32}H_{32}O_{10}S_2$ requires C, 60·0; H, 5·05; S, 10·0%).

The second crystalline product obtained from chromatography of methylated reaction product was recrystallised from methanol–ether to give (+)-*tetra*-O-*methyl*-6′-*phenylsulphonylcatechin* (2R,3S-configuration) (IV; R = Me, R′ = H) (0·30 g.) as needles, m. p. 192—193°, $[\alpha]_D^{24}$ +81·8° (*c* 0·5 in $CHCl_3$) (Found: C, 61·8; H, 5·45; S, 6·5. $C_{25}H_{26}O_8S$ requires C, 61·7; H, 5·4; S, 6·6%). The infrared spectrum in chloroform solution was identical with that of the racemic compound, and differed in Nujol by showing a less sharp hydroxyl absorption at 3500 cm.$^{-1}$.

(−)-3-O-*Acetyltetra*-O-*methyl*-6′-*phenylsulphonylcatechin* (2R,3S-configuration) (IV; R = Me, R′ = Ac) separated from ethyl acetate–ether as rods, m. p. 175—177°, $[\alpha]_D^{22}$ −37·8° (*c* 0·5 in $CHCl_3$) (Found: C, 61·5; H, 5·55; S, 6·45%). The infrared spectrum in Nujol was identical with that of the racemic acetate.

(±)-*Penta*-O-*acetyl*-6′-*phenylsulphonylcatechin* (IV; R = R′ = Ac.).—The crude amorphous sulphone (500 mg.) from the laccase-catalysed oxidation was warmed on a water-bath for 5 hr. with acetic anhydride (3·0 ml.) and dry pyridine (10 ml.). The product was isolated by pouring the reaction mixture into ice and dilute hydrochloric acid, followed by extraction with chloroform and evaporation of the solvent. Crystallisation from benzene afforded brown crystals (133 mg.) which were recrystallised several times from ethyl acetate to give the (±)-*penta*-O-*acetyl derivative* (113 mg.) as clusters of rods, m. p. 175·5—177·5° (Found: C, 57·8; H, 4·65; S, 5·75. $C_{31}H_{28}O_{13}S$ requires C, 58·1; H, 4·4; S, 5·0%); $\nu_{max.}$ (in Nujol) 1770 (broad, aromatic acetate C=O), 1750 (aliphatic acetate C=O), 1320 (sulphone), 1200 (broad, acetate C–O), and 1150 (sulphone) cm.$^{-1}$.

Laccase-catalysed Oxidation of (+)-*Catechin in Presence of Benzenesulphinic Acid*.—The procedure used was the same as that for (±)-catechin, with the exception that the reaction was kept at 25° for 6 days, a further quantity (15 ml.) of enzyme solution being added after 3 days. The colourless solution gradually turned a bright clear red, but on the addition of 2N-hydrochloric acid a yellow colour was produced. From (+)-catechin (1·5 g.), after methylation by the same procedure as before, chromatography on alumina, and recrystallisation from methanol–ether, (−)-*tetra*-O-*methyl*-6′-*phenylsulphonylcatechin* (2S,3R-configuration) (IV; R = Me, R′ = H) (0·66 g.) was obtained as needles, m. p. 190—192°, $[\alpha]_{}^{23}$ −81·2° (*c* 1·0 in $CHCl_3$) (Found: C, 61·45; H, 5·45; S, 6·35%).

Recrystallisation of a mixture of the (+)-tetramethylphenyl sulphone (15 mg.) and the (−)-tetramethylphenyl sulphone (15 mg.) from methanol–ether afforded (±)-tetramethylphenyl sulphone, m. p. and mixed m. p. 200–202°, $[\alpha]_D^{24}$ 0° (*c* 1·0 in $CHCl_3$).

(+)-3-O-*Acetyltetra*-O-*methyl*-6′-*phenylsulphonylcatechin* (2S,3R-configuration) (IV; R = Me, R′ = Ac.).—Obtained by acetylation of the (−)-tetramethylphenyl sulphone with acetic anhydride in pyridine, the *acetyl derivative* separated from ethyl acetate–ether as rods, m. p. 175—178°, $[\alpha]_D^{25}$ +35·4° (*c* 1·0 in $CHCl_3$) (Found: C, 61·05; H, 5·1; S, 6·4%).

Autoxidation of (±)-*Catechin in Presence of Benzenesulphinic Acid*.—Air was bubbled through solutions of (±)-catechin (0·50 g.) and sodium benzenesulphinate (0·5 g.) in 0·1M-phosphate buffer (150 ml.) of pH 8, 7, and 6, while the flasks were immersed in a bath at 35—40°.

(i) *At pH* 8. Red polymeric material soon began to separate and the absorption spectrum (in phosphate buffer) of a sample removed after 3 days showed a maximum at 420 mμ with a weak absorption at 275 mμ. After 3·5 days paper chromatography showed that no catechin remained in the solution, the only spot being at the origin. 2N-Hydrochloric acid (25 ml.) was added and the polymer (0·43 g.) was collected by centrifugation. Extraction of the aqueous solution with ethyl acetate afforded a negligible amount of material.

(ii) *At pH* 7. After 3·5 days the solution contained mostly unchanged catechin. After 7 days the solution was shown (paper chromatography) to contain catechin, sulphone, and polymeric material some of which had separated as a precipitate. After 9 days the solution contained mainly polymer with a little sulphone, but no unchanged catechin. 2N-Hydrochloric acid (25 ml.) was added and the polymer (0·39 g.) was collected by centrifugation. Extraction of the aqueous solution with ethyl acetate afforded a partly crystalline yellow gum which was methylated with

dimethyl sulphate and potassium carbonate in acetone. The methylated product was isolated in the usual way and chromatographed on alumina. Elution with benzene and crystallisation from methanol–ether afforded the (±)-tetramethyl sulphone (IV; R = Me, R′ = H) (9·2 mg.), m. p. 197—200°.

(iii) *At pH* 6. After 3·5 days the solution contained mostly unchanged catechin with a little of the sulphone (R_F 0·54). After 14 days the solution contained approximately equal quantities of catechin and sulphone with a trace of polymer. 2N-Hydrochloric acid (25 ml.) was added and a mixture of crystalline and brown amorphous material was separated. Warm methanol dissolved the crystalline product together with some of the amorphous product, and after the addition of water, pale brown needles separated in the cold, together with the amorphous polymer. Decantation removed most of the polymer, leaving the crystals (79 mg.) which were recrystallised from aqueous methanol (charcoal), affording needles (slow cooling) or plates (fast cooling), m. p. 164—168°. Paper chromatography (R_F 0·53) and infrared and ultraviolet comparisons (see above) showed that this was the (±)-sulphone (IV; R = R′ = H). Extraction of the aqueous solution with ethyl acetate afforded a yellowish-brown gum which was methylated with dimethyl sulphate and potassium carbonate in acetone. The methylated product was isolated in the usual way and chromatographed on alumina. Elution with benzene and crystallisation from methanol–ether afforded a mixture of sulphones with tetramethylcatechin (100 mg. of crystalline material). Fractional crystallisation afforded (±)-tetra-*O*-methylcatechin (44 mg.), m. p. 135—139°, (±)-tetra-*O*-methyl-6′-phenylsulphonylcatechin (25 mg.), m. p. 195—200°, and (+)-tetra-*O*-methyl-6′-phenylsulphonylcatechin (5 mg.), m. p. 189—192°.

Autoxidation of (±)-*Catechin at pH* 8.—The procedure was identical to that for the autoxidation in presence of benzenesulphinic acid. After 3 days the absorption spectrum (in the buffer solution) showed a maximum at 430 mμ with a weak absorption at 280 mμ. The red polymer (0·22 g.) was isolated by centrifugation.

Autoxidation of Catechol at pH 8.—Air was bubbled through a solution (A) of catechol (0·50 g.) in 0·1M-phosphate buffer of pH 8 (150 ml.), and through a similar solution (B) containing additionally sodium benzenesulphinate (0·50 g.), while the flasks were immersed in a bath at 30—35°. A dark grey sludge soon began to separate in each flask. After 3 days, paper chromatography showed unchanged catechol (R_F 0·75) and polymer (R_F 0) in solutions A and B, with also 4-phenylsulphonylcatechol (R_F 0·68; identical with the product from the laccase-catalysed oxidation of catechol in acetate buffer of pH 5[8]) in solution B. After a further 2 days paper chromatography indicated that most of the catechol had reacted. The polymeric material in flask A was isolated by centrifugation as an almost black powder (97 mg.).

Solution B was acidified with 2N-hydrochloric acid and extracted with ethyl acetate. The dark brown organic extract was dried, boiled with charcoal, filtered, and evaporated. The residue was crystallised from aqueous ethanol, and after a further recrystallisation from this solvent, and several hours at 110° *in vacuo*, afforded 4-phenylsulphonylcatechol (0·14 g.), identical with the laccase-catalysed oxidation product.

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