Plant Proanthocyanidins. Part I. Introduction; the Isolation, Structure, and Distribution in Nature of Plant Procyanidins

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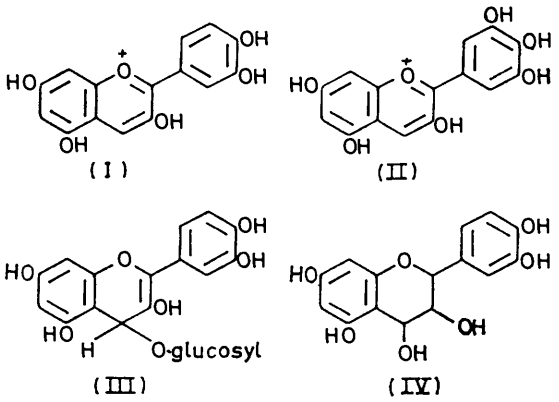
The isolation and determination of structure of some plant procyanidins in their free phenolic forms are described. The distribution of procyanidins in the tissues of twenty-nine plant species has been examined and the biogenetic implications of this study are briefly discussed.

THE development of deep red colours from colourless materials present in plant tissues upon treatment with mineral acid has long been known.[1-4] In 1920, Rosenheim,[5] when studying the anthocyanin pigments of the young grape vine (*Vitis vinifera*), isolated a colourless material which yielded cyanidin (I) on boiling with 10% hydrochloric acid. He named this new class of anthocyanidin progenitors ' leucoanthocyanins ' and suggested that they were glycosides of the pseudo-base of the corresponding anthocyanidin (III). Subsequently

[1] M. Tswett, *Biochem. Z.*, 1914, **58**, 225.
[2] K. Freudenberg and K. Weinges, *Bull. Nat. Inst. Sci. India*, 1965, **31**, 24.
[3] E. C. Bate-Smith and T. Swain, ' Chemistry of the Vegetable Tannins,' Soc. Leather Trades Chemists, Croydon, 1956, p. 109.
[4] R. Willstatter and T. J. Nolan, *Annalen*, 1915, **408**, 1.
[5] O. Rosenheim, *Biochem. J.*, 1920, **14**, 178.

Robinson and Robinson [6,7] and Bate-Smith and Lerner [8,9] examined the systematic distribution of ' leucoanthocyanins ' in plants and showed that the majority on treatment with acid gave rise to cyanidin (I) and the rest, with few exceptions, to delphinidin (II). Bate-



Smith [8,9] also correlated his observations with various botanical features and drew attention to the fact that ' leucoanthocyanins ' were confined mainly to the tissues of plants with a woody habit of growth. He also suggested that these substances are probably most commonly responsible for the broad range of reactions in plant tissues generally attributed to tannins. Fruit, for example, often contains substantial quantities of these substances which make important contributions to the quality, colour, and taste of the fruit and products derived therefrom. Various workers have since examined the chemical properties of the ' leucoanthocyanins ' and, contrary to Rosenheim's earlier suggestion, have concluded that two groups of substances, flavan-3,4-diols [*e.g.* (IV)] [10-12] and flavan-3-ol dimers and higher oligomers [*e.g.* (V)],[13-28] are principally responsible for the properties defined by the earlier work. Weinges [29] has proposed that these two groups of substances should be named respectively leucoanthocyanidins and proanthrocyanidins. This nomenclature is used here.

A number of racemic and some optically active leucocyanidins (IV) have been obtained from plants, but the determination of their stereochemistry and comparison with synthetically derived compounds has not been attempted.[30,31] On the other hand several procyanidin oligomers have been described [23-28] and Weinges and his colleagues have isolated,[15,16] as their acetates, four dimeric procyanidins [B-1—B-4 (V)—(VIII), Scheme 1]. Two further dimeric procyanidins (A-1 and A-2; $C_{30}H_{24}O_{12}$) in which two intermonomer linkages were postulated were also isolated by Weinges [15] and Mayer [17] and their colleagues. The structures proposed for the dimers B-1—B-4 [(V)—(VIII)] were based on ^1H n.m.r. and mass spectral data for the acetate and methyl ether derivatives, and on the release with acid of either (+)-catechin (IX) or (−)-epicatechin (X).

The wide distribution of procyanidins (Table 6) opens up some interesting questions of biosynthesis and possible

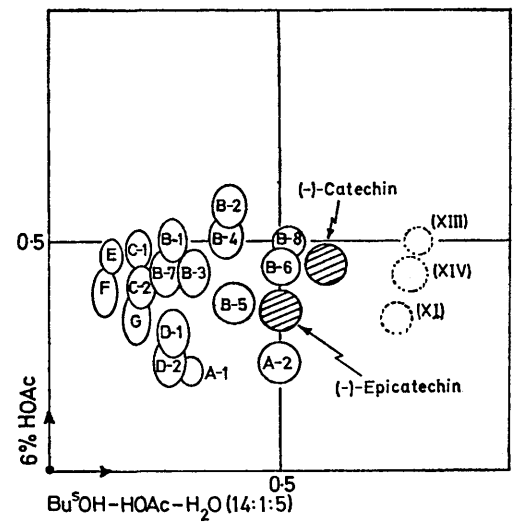
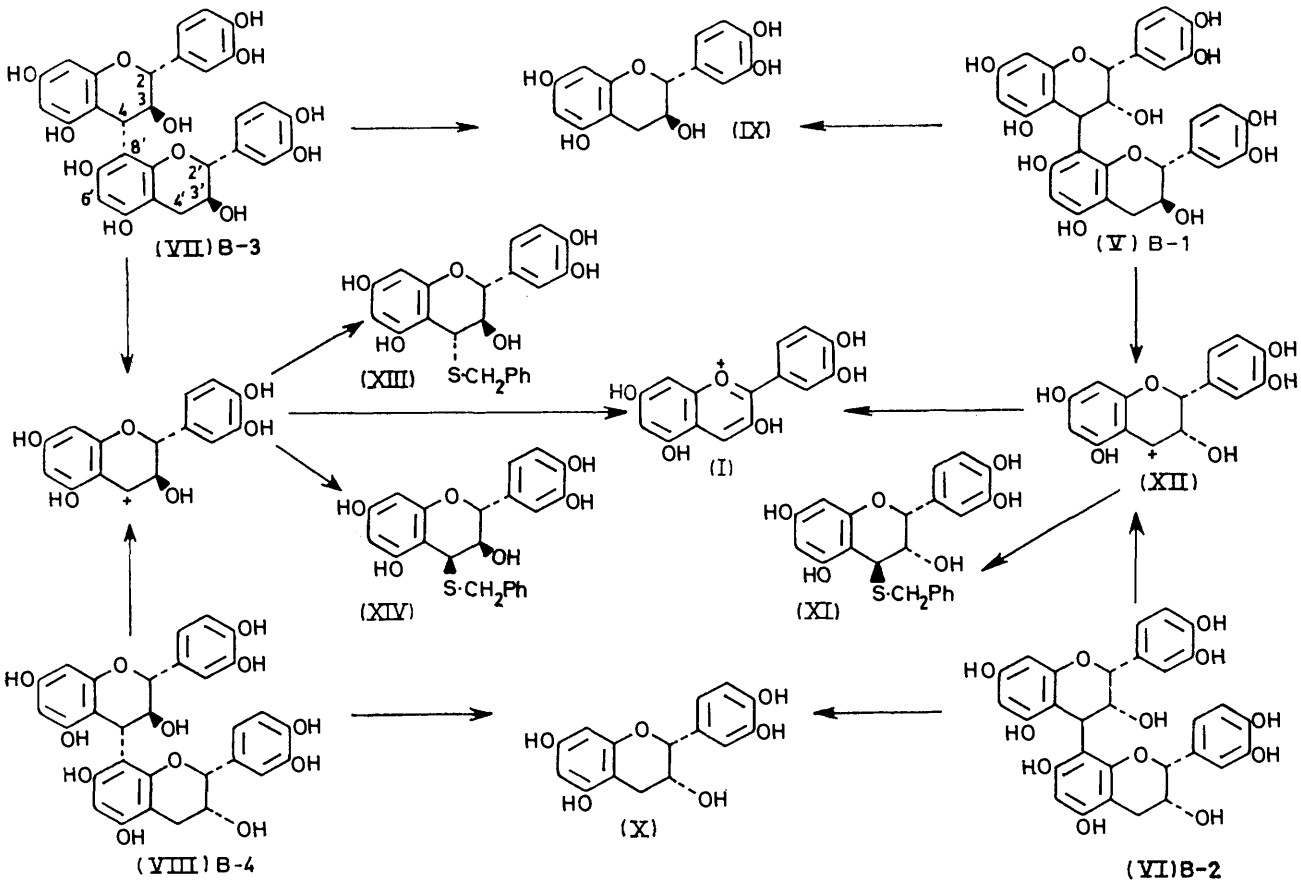


FIGURE 1 Paper chromatography of plant procyanidins

physiological function. As a necessary preliminary to such studies the dimers B-1—B-4 have been isolated and characterised in their free phenolic forms. Each dimer

[6] G. M. Robinson and R. Robinson, *J. Chem. Soc.*, 1935, 744.
[7] G. M. Robinson and R. Robinson, *Biochem. J.*, 1933, **27**, 206.
[8] E. C. Bate-Smith, *J. Exp. Bot.*, 1953, **4**, 1.
[9] E. C. Bate-Smith and N. H. Lerner, *Biochem. J.*, 1954, **58**, 126.
[10] D. G. Roux and S. E. Drewes, *Biochem. J.*, 1964, **90**, 343; 1964, **92**, 555; 1965, **94**, 482; 1966, **98**, 493.
[11] F. E. King and W. Bottomley, *J. Chem. Soc.*, 1954, 1399.
[12] J. W. Clark-Lewis and I. Dainis, *Austral. J. Chem.*, 1967, **20**, 2191.
[13] W. G. C. Forsyth, *Biochem. J.*, 1957, **65**, 177.
[14] W. G. C. Forsyth and J. B. Roberts, *Biochem. J.*, 1960, **74**, 374.
[15] K. Weinges, W. Kaltenhauser, H.-D. Marx, E. Nader, J. Perner, and D. Seiler, *Annalen*, 1968, **711**, 184.
[16] K. Weinges, K. Goritz, and F. Nader, *Annalen*, 1968, **715**, 164.
[17] W. Mayer, L. Goll, E. V. Arndt, and A. Mannschreck, *Tetrahedron Letters*, 1966, 429.
[18] S. E. Drewes, D. G. Roux, S. H. Eggers, and J. Feeney, *J. Chem. Soc.* (*C*), 1967, 1217.
[19] S. E. Drewes and A. H. Ilsley, *J. Chem. Soc.* (*C*), 1969, 897.
[20] S. E. Drewes, D. G. Roux, H. M. Saayman, S. H. Eggers, and J. Feeney, *J. Chem. Soc.* (*C*), 1967, 1302.
[21] I. C. du Preez, A. C. Rowan, and D. G. Roux, *Chem. Comm.*, 1970, 492.
[22] A. Pelter, P. I. Amenschi, R. Warren, and S. H. Harper, *J. Chem. Soc.* (*C*), 1969, 2572.
[23] M. J. Betts, B. R. Brown, P. E. Brown, and W. T. Pike, *Chem. Comm.*, 1967, 1110.
[24] K. D. Sears and R. L. Casebier, *Chem. Comm.*, 1968. 1437.
[25] T. A. Geissman and F. Dittmar, *Phytochemistry*. 1965, **4**, 267.
[26] V. C. Quesnel, *Phytochemistry*, 1968, **7**, 1583.
[27] E. Haslam, *J. Chem. Soc.* (*C*), 1969, 1824.
[28] A. G. Brown, W. B. Eyton, A. Holmes, and W. D. Ollis, *Nature*, 1969, **221**, 742.
[29] K. Weinges, W. Bahr, W. Ebert, K. Goritz, and H.-D. Marx, *Fortschr. Chem. org. Naturstoffe*, 1969, **27**, 158.
[30] M. I. Baig, J. W. Clark-Lewis, and M. J. Thompson, *Austral. J. Chem.*, 1969, **22**, 2645.
[31] M. J. Betts, B. R. Brown, and M. R. Shaw, *J. Chem. Soc.* (*C*), 1969, 1178.

was obtained as a colourless amorphous solid after fractionation of the plant extract on polyamide and Sephadex LH-20 dextran gel. The phenolic procyanidins were isolated from several plant sources (Table 1) and were characterised by analysis, paper chromatography (Figure 1), conversion into their deca-acetates,[16] ^{1}H n.m.r. spectroscopy (Figure 2), and, in some cases, by o.r.d. and c.d. measurements (Figure 3).



SCHEME 1 Acid-catalysed degradation of procyanidins

No structural conclusions were drawn from the o.r.d. and c.d. measurements owing to the absence of appropriate models; however the dimers B-3, B-4, and B-6 (see later), the trimer C-2 (see later), and their derivatives all showed negative plain dispersion curves with a small negative Cotton effect superimposed at around 275 nm. In contrast, approximately mirror image curves were displayed by the dimers B-1 and B-2.

Weinges, Marx, and Goritz [36] from a study of the temperature dependence of the ^{1}H n.m.r. spectra of some 4-arylpolymethoxyflavans concluded that there was restricted rotation at the C-4 (sp^3—sp^2) linkage, and they suggested, by analogy, that corresponding conformational isomers of the procyanidin dimers (B-1—B-4) might be found in nature. Preliminary evidence for such isomerism in the related cases of a trimeric profisetinidin and a dimeric proguibourtinidin (as their methyl ether acetates) has been obtained by Du Preez *et al.*[37] The ^{1}H n.m.r. spectra of the phenolic procyanidin dimers (B-1—B-4) fall into two categories. Those of B-1 (V) and B-2 (VI) are straightforward; first-order analysis is possible on the basis of pseudo-chair conformations for both hererocyclic rings (*e.g.* B-2, Figure 2) and in neither case was evidence of any conformational isomerism obtained. On the other hand the spectra of B-3 (VII) and B-4 (VIII), which were similar to each other (Figure 2), showed a multiplicity of signals. Both dimers were homogeneous as judged by paper chromatography and the spectra have been provisionally interpreted in terms of the presence of two conformers for each procyanidin. Satisfactory analysis follows (Table 2) if it is assumed that for both procyanidins B-3 and B-4 the heterocyclic ring in the 'lower' flavan-3-ol adopts a skew-boat conformation, with the aryl group at C-2′ in a quasiaxial position, and

[32] H. W. H. Schmidt and H. NeuKom, *J. Agric. Food Chem.*, 1969, **17**, 344.
[33] W. G. C. Forsyth, *Biochem. J.*, 1955, **60**, 108.
[34] E. Haslam and J. Jaggi, *Phytochemistry*, 1969, **8**, 635.
[35] T. Swain and L. L. Creasey, *Nature*, 1965, **208**, 151.
[36] K. Weinges, H.-D. Marx and K. Goritz, *Chem. Ber.*, 1970, **103**, 2336.
[37] I. C. du Preez, A. C. Rowan, D. C. Roux, and J. Feeney, *Chem. Comm.*, 1971, 315.

that for both dimers the same phenomenon is responsible for the complexity of the spectra. The methylene signal from the dimer B-3 shows two identical pairs of octets and for the deca-acetate of the dimer B-4 the H-4 signal appears as two identical triplets. These observations indicate that the coupling constants $J_{3,4}$, $J_{3',4'\text{-}pro\text{-}R}$ and $J_{3',4'\text{-}pro\text{-}S}$ are the same in both conformers, and on this basis their existence is, at this stage, tentatively interpreted in terms of restricted rotation about the

TABLE 1

Isolation of plant procyanidins *

B-1 (−)-Epicatechin–(+)-catechin	*Malus sylvestris* (crab apple) [b]
	Pyrus malus (apple) [32,b]
	Vaccinium vitis-idaea (mountain cranberry) [15,16,a]
	Persea gratissima (avocado pear stone) [25,35,a]
	Theobroma cacao (unripe cocoa bean) [13,14,33,b]
	Aesculus hippocastanum (horse chestnut, seed shell) [17,b]
	Crataegus monogyna (hawthorn, fruit) [15,b]
B-2 (−)-Epicatechin$_2$	*M. sylvestris* [a]
	P. malus [a]
	P. gratissima [a]
	T. cacao [a]
	A. hippocastanum [a]
	C. monogyna [a]
	V. vitis-idaea [a]
B-3 (+)-Catechin$_2$	*V. vitis-idaea* [b]
	Salix caprea (goat willow, catkin) [34,a]
	Fragaria x anannasa (unripe strawberry) [35,a]
B-4 (+)-Catechin–(−)-Epicatechin	*Rubus fructicosus* (blackberry, unripe fruit, stem) [a]
	Rubus idaeus (raspberry, unripe fruit, stem) [a]
B-5 (−)-Epicatechin$_2$	*T. cacao* [c]
	A. hippocastanum [b]
B-6 (+)-Catechin$_2$	*S. caprea* [c]
	Picea abies (young shoots) [c]
B-7 (−)-Epicatechin–(+)-catechin	*V. vitis-idaea* [d]
	S. caprea [d]
B-8 (+)-Catechin–(−)-epicatechin	*R. fructicosus* [d]
	R. idaeus [d]
C-1 (−)-Epicatechin$_3$	*T. cacao* [d]
C-2 (+)-Catechin$_3$	*S. caprea* [d]

* The amounts of procyanidins isolable vary with the maturity of the plant tissue and the figures quoted refer to freshly obtained, unripe tissue: [a] 0·1—0·5%; [b] 0·05—0·1% [c] 0·01—0·05%; [d] <0·01%.

6′,4-(or 8′,4-)linkage rather than ' flipping ' of one of the heterocyclic rings.

Variable-temperature studies reinforced the view that the presence of two conformers is responsible for the complexity of the spectra. Preliminary observations at 100 MHz show, for example in the case of the dimer B-4 and its deca-acetate (in [$^2H_6$]dimethyl sulphoxide and [$^2H_5$]pyridine), that increase in temperature causes simultaneous and progressive coalescence of the duplicated aromatic proton signals until this region closely resembles that in the spectra of the dimers B-1 and B-2. The original spectrum is reconstituted on cooling. The temperature of coalescence is *ca.* 75 °C, which supports the suggestion that these conformational isomers would

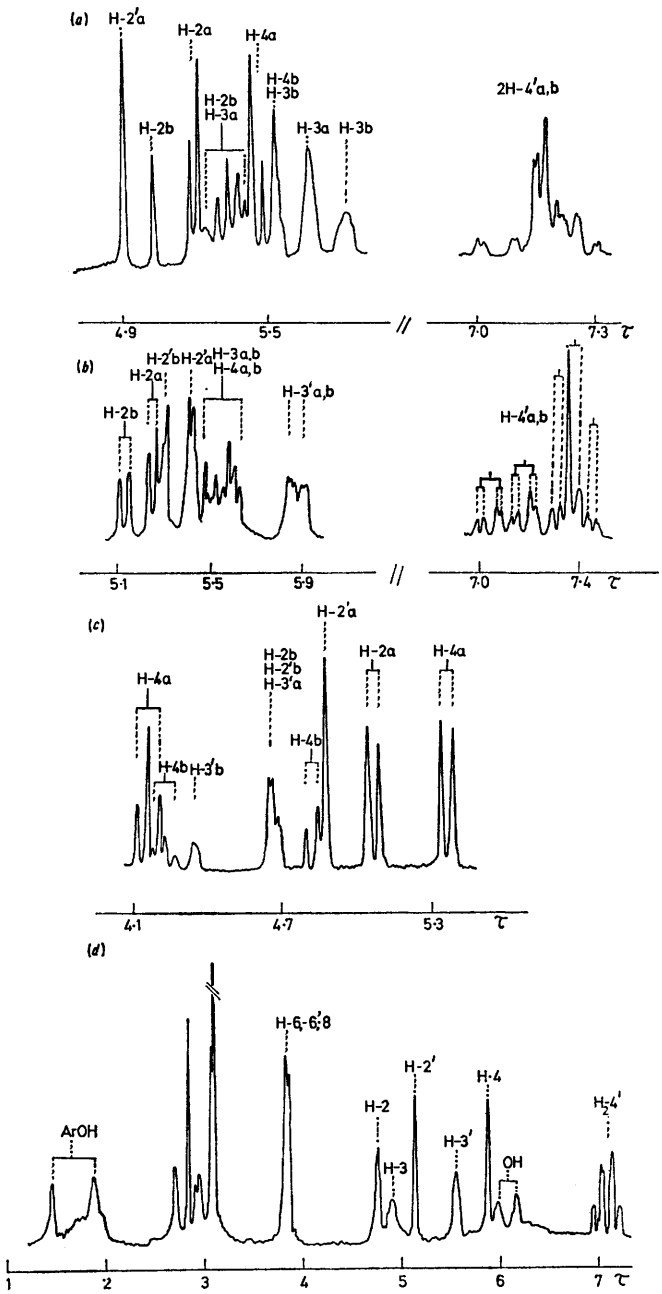


FIGURE 2 ^{1}H N.m.r. spectra (220 MHz) of phenolic procyanidins (τ values; $SiMe_4$ standard); (*a*) B-4 (VIII) in [$^2H_6$]acetone, (*b*) B-3 (VII) in [$^2H_6$]acetone, (*c*) B-4 (VIII) deca-acetate in [^2H]chloroform, (*d*) B-2 (VI) in [$^2H_6$]acetone

not be separable at room temperature.[38] It is perhaps significant that this behaviour has only been observed in dimers B-3 and B-4 which have (+)-catechin as the ' upper half ' of the dimer; this suggests that for restricted rotation about the interflavan linkage a third

[38] W. A. Thomas in ' Annual Review of N.M.R. Spectroscopy,' ed. E. F. Mooney, Academic Press, London, 1968, p. 43.

condition, in addition to those proposed by Weinges,[36] is necessary, *i.e.* that the ' upper half ' of the dimer should have a 2,3-*trans*-configuration with the hydroxy-group at position 3 in a quasiequatorial position in the heterocyclic ring.

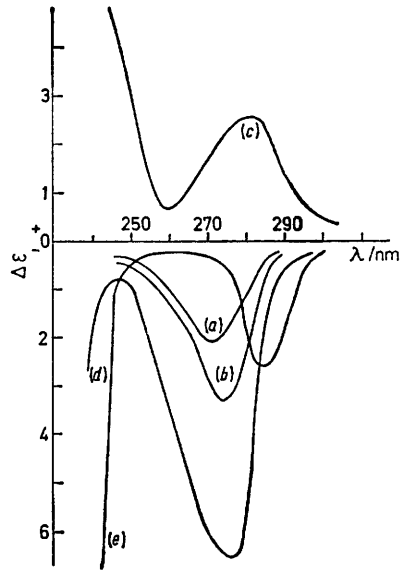


FIGURE 3 C.d. measurements on procyanidins; (*a*) (−)-epicatechin penta-acetate, (*b*) (+)-catechin penta-acetate, (*c*) B-1 (V) deca-acetate, (*d*) B-4 (VIII) deca-acetate, (*e*) B-3 (VII)

detected by paper chromatography immediately after the commencement of this reaction, but cyanidin (I) cannot. Unidentified substances ($\lambda_{max.}$ 450 nm) are first formed which finally yield cyanidin on further heating in the presence of oxygen. Jurd and Somers [40] have suggested that xanthylium salts are also formed in this reaction but evidence to support this view was not obtained. At 20° in the absence of oxygen the procyanidins are degraded by acid to the flavan-3-ol and polymeric materials (phlobaphens, $\lambda_{max.}$ 400—450 nm) and do not give cyanidin (I). This general behaviour with acid contrasts with that of dimers of the *A* type,[15,17] which appear to give cyanidin directly upon heating in acid, although under comparable conditions much more slowly.

Specific cleavage of the monomer–monomer linkage in the phenolic procyanidins (V)—(VIII) was achieved in high yield by reaction with toluene-α-thiol and acetic acid in ethanol. Thioacetic acid [23,24,41] in ethanol smoothly cleaved the procyanidin dimers (V)—(VIII) in *ca.* 30 min to give compound (IX) or (X). However the other phenolic products of this reaction were not readily analysed or separated and the cleavage was conducted alternatively with toluene-α-thiol and acetic acid. The reaction was much slower (24—36 h) but the products were all amenable to analysis and separation. The dimers B-1 and B-2 [(V) and (VI)] gave (+)-catechin (IX) and (−)-epicatechin (X), respectively, and one sulphur-containing compound which is formulated as

TABLE 2

Procyanidin dimers (220 MHz spectra: τ values; *J* in Hz; $SiMe_4$ standard)

Dimer	ArOH	ArH Ring A	ArH Ring B	OH	2-H	3-H	4-H	2′-H	3′-H	4′-H_2
B-1 [a]	1·5—2·5(m)	3·9—4·1(m)	2·8—3·4(m)	6·2—6·5(m)	5·20(s)	5·65br(s)	5·90(s)	4·95(d) (*J* 8)	4·80(m)	7·0—7·3(m)
B-2 [a]	1·5—2·2(m)	3·85br(s)	2·7—3·15(m)	5·95br(s), 6·18br(s)	5·13(s)	5·55(s)	5·89(s)	4·75(s)	4·90br(s)	7·0(dd), 7·14(d) (*J* 3 and 14)
B-5 [a]	1·5—2·3(m)	3·90br(s)	1·95—3·30(m)	6·15br(s), 6·35br(s)	5·19(s)	5·80br(s)	5·90(s)	5·05(s)	5·36br(s)	7·2—7·4(m)
B-3 [a]										
Conformer (a)	1·5—2·5(m)	2·65—4·2(m)		5·9—6·2(m)	5·27(d) (*J* 8·0)	5·45—5·71(m)	5·65(d) (*J* 9·5)	5·42(d) (*J* 3·5)	5·90(m)	6·8—7·4(m)
Conformer (b)					5·15(d) (*J* 8·0)	5·45—5·71(m)	5·50(d) (*J* 9·5)	5·31(d) (*J* 3·5)	5·83(m)	
B-4 [a]										
Conformer (a)	1·4—2·6(m)	2·60—4·15(m)		5·9—6·3(m)	5·20(d) (*J* 8·0)	5·32(m)	5·45(d) (*J* 10·0)	4·91(s)	5·67br(s)	6·98—7·30(m)
Conformer (b)					5·40(d) (*J* 8·0)	5·52(m)	5·52(m)	5·04(s)	5·81br(s)	
B-4 acetate [b]										
Conformer (a)		2·40—3·50(m)			5·10(d) (*J* 8·5)	4·15(t)	5·36(d) (*J* 8·5)	4·90(s)	4·67(m)	6·80—7·40(m)
Conformer (b)					4·71(d) (*J* 8·5)	4·21(t)	4·88(d) (*J* 8·5)	4·65(s)	4·35(m)	

[a] Solution in $(CD_3)_2CO$. [b] Solution in $CDCl_3$.

Treatment of the individual procyanidins with ethanolic hydrochloric acid at 60° gave cyanidin (I) (25—30% yield based [39] on absorption at 535 nm) and either (−)-epicatechin (X) [from B-2 (VI) and B-4 (VIII)] or (+)-catechin (IX) [from B-1 (V) and B-3 (VII)]. The free flavan-3-ol (IX) or (X) may be (2*R*,3*S*,4*S*)-4-benzylthioflavan-3,3′,4′,5,7-pentaol (XI). Under acid catalysis the free flavan-3-ol is presumed to be derived from the ' lower half ' of the dimer and the transient carbonium ion (XII) from the ' top half '; the ion (XII) is then captured stereospecifically to give the thioether (XI). Analysis of the ^1H n.m.r. spectra of

[39] J. B. Harborne, ' Comparative Biochemistry of the Flavanoids,' Academic Press, London and New York, 1967, p. 6.

[40] L. Jurd and T. C. Somers, *Phytochemistry*, 1970, **9**, 419.

[41] B. O. Lindgren, *Acta Chem. Scand.*, 1950, **4**, 1365.

TABLE 3

(2*R*,3*S*,4*S*)-4-Benzylthioflavan-3,3′,4′,5,7-pentaol (XI) and (−)-epicatechin (X) (100 MHz spectra; τ values; $SiMe_4$ standard; *J* in Hz)

	2-H	3-H	4-H	6-H	8-H	2′-, 5′-, 6′-H	Ph	S·CH_2	OH	OMe	OAc
(X) [a]	5·16(s)	5·81(m)	7·14—7·32(m)	4·10(d) (*J* 2·5)	4·01(d)	2·98(s), 3·24(s)			5·9—6·1(m)		
(XI) [a]	4·73(s)	6·00(m)	5·88(d) (*J* 3·0)	4·10(d) (*J* 2·0)	3·99(d)	2·94(s), 3·22(s)	2·5—2·85(m)	6·00(d), 6·10(d) (*J* 14·0)	6·4—6·6(m)		
Methyl ethers:											
(X) [b]	5·09(s)	5·75(m)	7·04—7·15(m)	3·91(d) (*J* 2·5)	3·85(d)	2·95(s), 3·20(s)			7·24(d) (*J* 6·0)	6·13(s), 6·15(s), 6·25(s), 6·28(s)	
(XI) [b]	4·60(s)	6·10—6·30(m)	5·97(d) (*J* 2·0)	3·95(d) (*J* 2·0)	3·89(d)	3·05(s), 3·25(s)	2·65—2·85(m)	6·10—6·30(m)	8·15(m)	6·10(s) 6·30(3me,s)	
Acetates:											
(X) [b]	4·92(s)	4·60(m)	7·00—7·12(m)	3·45(d)	3·31(d)	2·60(s), 2·80(s)					7·74(s), 8·10(s
(XI) [b]	4·38(s)	4·82(d) (*J* 1·0)	5·92(d)	3·55(d) (*J* 2·0)	3·40(d)	2·6—2·9(m)	2·6—2·9(m)	6·0(d), 6·25(d) (*J* 15·0)			7·81(s), 7·85(s) 8·16(s), 8·26(s)

[a] Solution in $(CD_3)_2CO$. [b] Solution in $CDCl_3$.

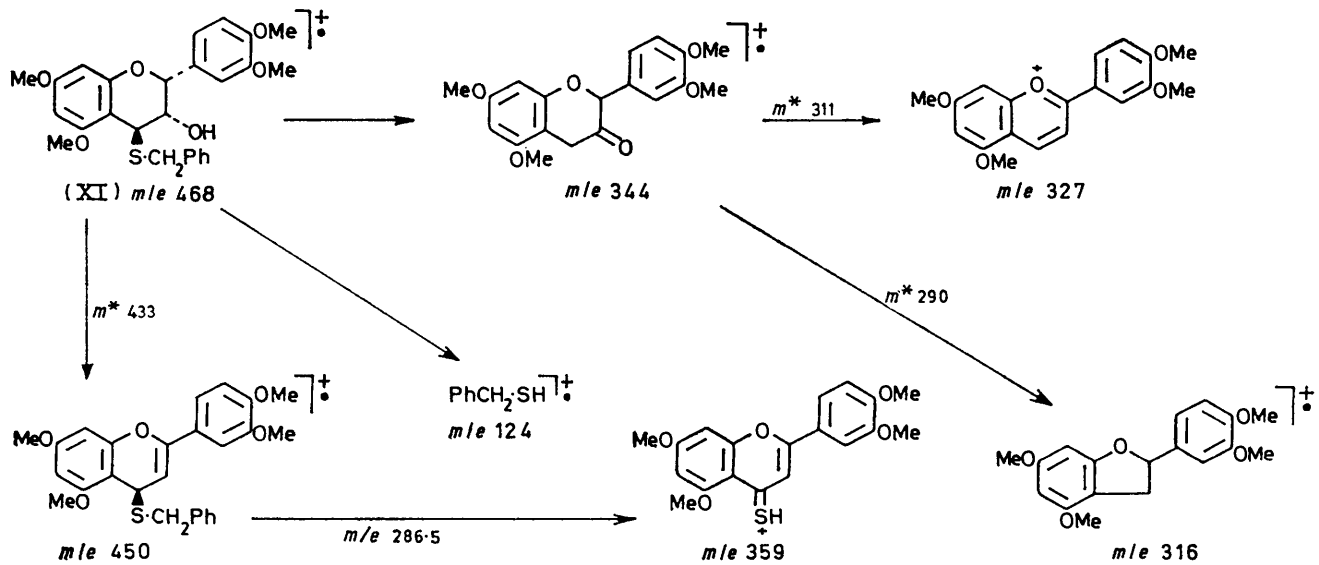
TABLE 4

(2*R*,3*R*,4*S*)-4-Thiobenzylflavan-3,3′,4′,5,7-pentaol (XIV) and (+)-catechin (IX) (100 MHz spectra; τ values; $SiMe_4$ standard; *J* in Hz)

	2-H	3-H	4-H_2 *		6-H †	8-H †	1′-, 5′-, 6′-H	Ph	S·CH_2	OH	OMe	OAc
(IX) [a]	5·41(d) ($J_{2,3}$ 8·0)	5·94(m)	7·46(dd) ($J_{3,4}$ 8·0)	7·08(dd) ($J_{3,4}$ 5·0)	4·12(d)	3·98(d)	3·11(s), 3·24(s)			5·8—6·0(m)		
(XIV) [a]	5·05(d) ($J_{2,3}$ 9·5)	5·75(dd) ($J_{3,4}$ 4·0)		5·58(d) ($J_{3,4}$ 4·0)	4·19(d)	3·94(d)	3·03(s), 3·18(s)	2·55—2·85(m)	5·9(s)	6·1—6·4(m)		
(XI1) [a]	5·52(d) ($J_{2,3}$ 7·5)	5·8—6·1(m)	6·16(d) ($J_{3,4}$ 9·0)		4·05(d)	3·99(d)	3·12(s), 3·24(s)	2·55—2·85(m)	5·8—6·2(m)	6·2—6·5(m)		
Methyl ethers:												
(IX) [b]	5·40(d) ($J_{2,3}$ 8·0)	6·05(m)	7·52(dd) ($J_{3,4}$ 8·5)	7·00(dd) ($J_{3,4}$ 4·5)	3·91(d)	3·87(d)	3·09(s), 3·15(s)			7·0—7·2(m)	6·11(s), 6·17(s), 6·25(s), 6·29(s)	
(XIV) [b]	5·18(d) ($J_{2,3}$ 10·0)	5·85(dd) ($J_{3,4}$ 4·0)		5·62(d) ($J_{3,4}$ 4·0)	3·90(d)	3·85(d)	2·9(s), 3·15(s)	2·6—2·8(m)	5·85(d), 6·05(d) (*J* 15·0)	7·10(d) (*J* 6·0)	6·07(6H,s), 6·17(s), 6·23(s)	
Acetates:												
(IX) [b]	4·62(d) (*J* 8·5)	4·75(m)	7·52(dd) ($J_{3,4}$ 7·0)	7·10(dd) ($J_{3,4}$ 5·5)	3·41(d)	3·33(d)	2·7(s), 2·85(s)					7·75(9H,s), 8·02(s)
(XIV) [b]	4·58br(s)	4·58br(s)		5·46(d) ($J_{3,4}$ 3·0)	3·40(d)	3·36(d)	2·65—2·85(m)	2·65—2·85(m)	6·04(d), 6·16(d), (*J* 15·0)			7·72(s), 7·77(s) 7·97(s)

[a] Solution in $(CD_3)_2CO$. [b] Solution in $CDCl_3$.

* J_{gem} 16·0 Hz throughout. † $J_{6,8}$ 2·0 Hz throughout.

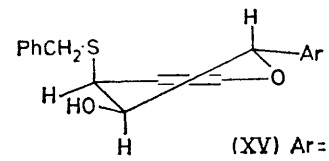


SCHEME 2 Mass spectral fragmentation of (2*R*,3*S*,4*S*)-4-benzylthioflavan-3,3′,4′,5,7-pentaol (XI)

compound (XI) and its derivatives (Table 3) does not permit a direct assignment of the 3,4-*trans*-configuration but the spectra are notable for the deshielding of H-2 relative to H-2 in the corresponding (−)-epicatechin derivatives. This is most readily explicable if it is assumed that the benzylthio-group occupies the quasi-axial [42] 4-*pro*-*S*-position in the heterocyclic ring; the deshielding of H-2 is then due to the sulphur atom in the 1,3-diaxial relationship. Chemical analogy provides additional support for this assignment. It has been shown [31,43,44] that solvolysis of 2,3-*cis*-flavan-3,4-diols, yields, by an *S*−1 mechanism, 4-ethers which have exclusively the 3,4-*trans*-stereochemistry. Participation of the neighbouring axial 3-hydroxy-group is believed to control the stereochemistry of these reactions. Additional proof for the structure (XI) for the thioether was derived from its smooth transformation into cyanidin (I) with acid and into (−)-epicatechin (X) after treatment with Raney nickel. Further support for structure (XI) was obtained from the mass spectrum of the methyl ether, which showed similarities to those of related flavan-3,4-diols,[45,46] although the normal retro-Diels–Alder reaction does not appear to play a significant role in the decomposition. The principal fragment ions derived from the thioether (XI) (Scheme 2) are formulated in accordance with earlier related work.[45-47]

The procyanidin dimers B-3 (VII) and B-4 (VIII), when treated similarly with toluene-α-thiol gave (+)-catechin (IX) and (−)-epicatechin (X), respectively, and two thioethers [(XIII) and (XIV)], both of which gave cyanidin (I) with acid and (+)-catechin (IX) with Raney nickel. The formation of two thioethers in this reaction is again supported by analogous work [43,44] on the solvolysis of 2,3-*trans*-flavan-3,4-diols. These, in contrast to 2,3-*cis*-flavan-3,4-diols, give mixtures of the 3,4-*trans*- and 3,4-*cis*-ethers, the composition of which is controlled by thermodynamic factors. The major thioether product is formulated on the basis of analogous mass spectra and ^{1}H n.m.r. data (Table 4) as the 2*R*,3*R*,4*S*-isomer (XIV), with the thioether group in the quasiaxial 4-position. The minor isomer, in which H-2 is no longer deshielded relative to the parent flavan-3-ol (IX) (Table 4) is assigned the alternative 2*R*,3*R*,4*R*-structure (XIII). The ^{1}H n.m.r. characteristics of the parent phenol (XIV) and methyl ether are consistent with the adoption by the heterocyclic ring of conformation (XV), and the sharp doublet for the 3-hydroxy-group in the methyl ether suggests that this may be stabilised by some form of hydrogen bonding, possibly to the sulphur atom. The penta-acetate of (XIV) probably adopts a similar conformation, but the apparent magnetic equivalence of H-2 and H-3 in the 100 MHz spectrum does not permit an unequivocal assignment.

The dimers B-1—B-4 are formulated as (V)—(VIII) in accordance with the suggestion of Weinges [15] that the linkage to ring A of the 'lower' flavan-3-ol unit was to the 8'- rather than to the 6'-position, although the latter was not specifically excluded. This proposal was made



(XV) Ar = $C_6H_3(OH)_2$-3,4

on chemical and biogenetic grounds and for the dimer B-3 has been confirmed in a synthesis of the diacetyl-octamethylprocyanidin.[48] Attempts to utilise the specific cleavage reaction with toluenethiol to provide chemical evidence for the position of the dimer linkage failed. Thus treatment of the dimer B-2 (VI) with toluene-α-[^{2}H]thiol gave (−)-epicatechin and the thioether (XI), in both of which approximately one atom of deuterium (^{1}H n.m.r. and mass spectral data) was substituted randomly in the two free positions (6 and 8) in ring A. Similar products resulted from the reaction of (−)-epicatechin and (XI) with toluene[^{2}H]thiol, and the reaction may thus be useful as a means of isotopic labelling of the phloroglucinol ring A of this type of flavan-3-ol.

The cleavage reaction thus provides a simple procedure for the isolation and identification of both monomer units of procyanidin dimers of the type B-1—B-4, but it is significant that the dimer A-2 [17] is recovered unchanged from this reaction and the procyanidins D-1 and D-2 (Figure 1) react very slowly. The paper chromatographic characteristics of the thio-ethers [(XI), (XIII), and (XIV); Figure 1] permit the reaction to be used for procyanidin identifications on a semimicro-scale and in situations where a high incidence of rotational isomerism about the interflavanoid bond does not permit extensive use of ^{1}H n.m.r. spectroscopy. It has been utilised thus to elucidate the constitution of the procyanidins B-5, B-6, B-7, and B-8, and C-1 and C-2 (Table 1), which are only available in small quantity. The dimers B-5, B-6, B-7, and B-8 have been established as isomers of B-2, B-3, B-1, and B-4, respectively, on the basis of the products of reaction with toluenethiol (Table 5). The

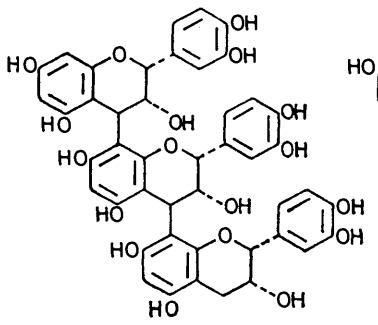
TABLE 5

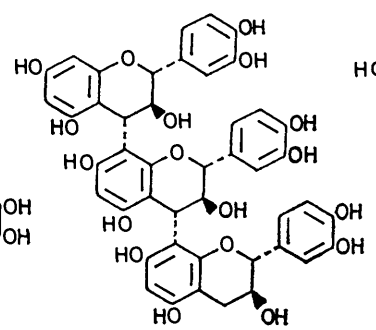
Reaction of procyanidins with toluene-α-thiol

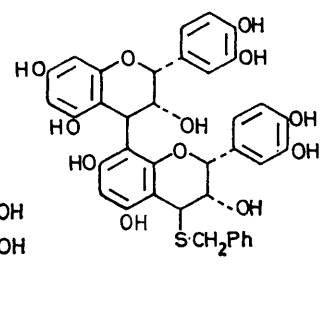
Procyanidin	Products
B-5	(−)-epicatechin, (XI)
B-6	(+)-catechin, (XIII), (XIV)
B-7	(+)-catechin, (XI)
B-8	(−)-epicatechin, (XIII), (XIV)
C-1	(−)-epicatechin, B-2, (XI), (XVIII)
C-2	(+)-catechin, B-3, (XIII), (XIV), (XIX)

[42] J. W. Clark-Lewis, *Austral. J. Chem.*, 1968, **21**, 2059.
[43] J. W. Clark-Lewis and L. R. Williams, *Austral. J. Chem.*, 1967, **20**, 2151.
[44] I. C. duPreez, D. Ferreira, and D. G. Roux, *J. Chem. Soc.* (*C*), 1971, 336.
[45] J. W. Clark-Lewis, *Austral. J. Chem.*, 1968, **21**, 3025.
[46] S. E. Drewes, *J. Chem. Soc.* (*C*), 1968, 1140.
[47] B. Willhalm, A. F. Thomas, and F. Gautschi, *Tetrahedron*, 1964, **20**, 1185.
[48] K. Weinges, J. Perner, and H.-D. Marx, *Chem. Ber.*, 1970, **103**, 2344.

isomerism may be structural (position of linkage to ' lower ' flavan-3-ol unit) or stereochemical (at position 4 of ' upper ' flavan-3-ol). In the case of all the dimers further limitations are set by the ^1H n.m.r. spectra (*e.g.* dimer B-5, Table 2) which show that, as with the isomers B-1—B-4, linkage is to the 6- or 8-position of the ' lower ' flavan-3-ol. The isolation of these further four identified along with a further compound, formulated as the thioether of B-2 (XVIII) on the basis of its paper chromatographic relationship to B-2. In an entirely analogous manner C-2 reacted with toluenethiol to give (+)-catechin and the thioethers (XIII and (XIV); at an intermediate stage of the solvolysis the dimer B-3 (VII) and the thioether (XIX) were detected.


(XVI) C-1


(XVII) C-2


(XVIII)

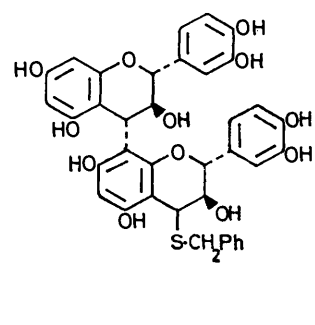

(XIX)

TABLE 6

Plant procyanidins—distribution

	(+)-Catechin	(−)-Epicatechin	(+)-Gallocatechin	(−)-Epigallocatechin	B-1	B-2	B-3	B-4	B-5	B-6	B-7	B-8	C-1	C-2	A-1	A-2	D-1	D-2	E	F	G
Rubus fructicosus [a,b,d]	(+)	+ *						+ *	+			+									†
Rubus idaeus [a,b,d]	(+)	+ *						+ *	+			+									†
Malus sylvestris [a,b]	+	+ *			+ *	+ *		+	+				+			+	(+)		+		†
Sorbus aucuparia [b]		+ *			+ *	+ *							+						+		
Cotoneaster horizontalis [a,b]	+	+ *			+ *	+ *		+	+				+				(+)		+		†
Crataegus monogyna [a,b]	(+)	+ *			+ *	+ *		+	+				+				(+)		+		†
Viburnum burkwoodii [a]	+	+ *			+ *	+ *		+	+				+						+		
Theobroma cacao [b,c]	+	+			+ *	+ *	(+)	(+)	+				+ *						+		†
Persea gratissima [a,b]	(+)	+ *			+ *	+ *							+						+		†
[c]	+ *	+ *			+ *	+ *	+	+	+				+						+		†
Camellia japonica [a]	(+)	+ *			+	+ *	+	+	+				+								
Aesculus hippocastanum [a,b]	(+)	+ *			+ *	+ *	+	+	+				+		+	+ *	+	+	+		†
Vaccinium myrtillus [a]	+	+ *		+ *	+ *	+ *		(+)					+		(+)						
Vitis vinifera [b]	+ *	+ *			+ *	+ *	+	+													†
Calluna vulgaris [a]	+ *	+ *			+ *		+	+	+								+				
Ribes sanguineum [a]	+	+ *			+	+								+			+ *				
Azalea indica [a]	+ *	+ *			+	+							+				+ *				
Prunus cerasus [b]	+	+ *			+ *	+ *			+				+						+		
Vaccinium vitis-idaea [a,b]	+ *	+			+ *	+	+ *	+	(+)	+		+		+	+ *	+ *		+				
Salix caprea [a,c]	+ *				+		+ *			+		+		+ *							+
Salix viminalis [a]	+ *				+		+ *			+		+		+ *							+†
Rosa rubiginosa [a]	+ *				+ *																
Fragaria x anannasa [a,b]	+ *				+		+ *			+				+ *							+†
Rhodendron ponticum [a]	+ *						+			+ *		+		+			+ *				+
Populus canescens [a,e]	+ *				+ *		+ *			(+)				+						+	
Quercus robur [a,b]	+ *				+ *		+ *			(+)		(+)		(+)							
Quercus petraea [a,b]	+ *				+ *		+ *			(+)	(+)		(+)								
Pinus sylvestris [a]	+ *		+ *		+		+			+ *				+							
Picea abies [a]	+ *		+ *		+	(+)	+	(+)		+ *				+							†

\+ Present; + * major component; (+) minor component. † Major procyanidins isolated.

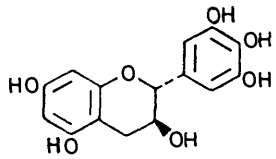
[a] Leaf; [b] fruit/skin; [c] seed; [d] stem; [e] catkins.

dimers is of biosynthetic significance, since it points to the possibility of the controlling process in procyanidin formation being chemical rather than enzymic in origin.

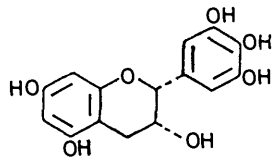
The procyanidins C-1 and C-2 were shown by mass spectroscopy of their methyl ethers to be trimeric ($M^{\cdot+}$ 1034) and they have been provisionally formulated as (XVI) and (XVII), respectively. Treatment of C-1 with toluenethiol gave the thioether (XI) and (−)-epicatechin. During the reaction the dimer B-2 (VI) was

As part of a programme of biosynthetic studies the earlier surveys of Robinson and Robinson [6,7] and Bate-Smith and Lerner [9] have been elaborated, and procyanidin distribution has been correlated with that of (+)-catechin and (−)-epicatechin in the living tissues of twenty-nine plant species (Table 6). The analysis was carried out in a number of cases by isolation and comparison with authentic samples, but more generally by two-dimensional paper chromatography. The re-

corded patterns of occurrence of the procyanidins (Table 6) refer, except where otherwise indicated, to phenols which may be readily detected in the plant extract. However in all cases where enrichment of the extract was achieved during isolation it was invariably observed that small amounts of additional procyanidins were present which were not detected in the crude extract. In many of the examples studied one of the diastereoisomeric flavan-3-ols, (+)-catechin (IX) or (−)-epicatechin (X), clearly predominates in the plant and occasionally to the apparent exclusion of the other isomer. In a parallel manner a distinctive paper chromatographic pattern of procyanidins is also revealed. Thus when there is a predominance of (+)-catechin there is also a preponderance of the dimers B-1 and B-3 and the trimer C-2. Alternatively where (−)-epicatechin predominates so also do the dimers B-1, B-2, and B-4 and the trimer C-1; the most frequently recognised pattern is the co-occurrence of (−)-epicatechin with B-1, B-2, and C-1. In no case was (+)-catechin or (−)-epicatechin found without the simultaneous detection of one or more of the procyanidin dimers and in general similar patterns of distribution were found in different tissues of the same plant. For some plants the pattern of procyanidins is complicated owing to the additional presence of other proanthrocyanidins. Typical of this category are those containing (+)-gallocatechin (XX) or (−)-epigallocatechin (XXI)



(XX)



(XXI)

as well as (+)-catechin and (−)-epicatechin. In these cases (*e.g. Picea abies*, *Pinus sylvestris*, and *Vaccinium myrtillus*) mixed dimers and oligomers, which yield cyanidin or delphinidin (II) on treatment with acid, also occur.

In all the examples studied the flavan-3-ols (IX) and (X) and related procyanidins were always found free, not glycosylated, and this points to the probability of these compounds being actively metabolised in living plant tissues. Much recent work, particularly with models, has favoured the proposal of a simulated acid-catalysed condensation of the related flavan-3,4-diols as a mode of biosynthesis of the procyanidins,[3,10,18,20,25,49-51] but in close examination of several plants [Table 6 (†)] no monomers of this class have been identified. This observation, in agreement with some earlier studies,[15,16,27] supports the alternative hypothesis [15,27,52,53] that the procyanidins may be formed by oxidative polymerisation of flavan-3-ol precursors. Preliminary experiments have shown the feasibility of this mode of biogenesis. [*u*-^{14}C]-(−)-Epicatechin was prepared by administration of [*u*-^{14}C]glucose to young shoots of *Rubus idaeus* or *Crataegus monogyna* or by exposure of such tissues to $^{14}CO_2$ under illumination. When labelled (−)-epicatechin was subsequently fed to shoots of *R. idaeus*, the dimer B-4 (VIII) was isolated after seven days and showed incorporation of 14% of the original activity administered. The biogenetic implication of these observations is currently under examination.

EXPERIMENTAL

Mass spectra were obtained with A.E.I. MS 9 and 12 instruments. ^{1}H N.m.r. spectra were recorded at 60 MHz and 100 MHz with Varian A-60 and HA-100 spectrometers, and at 220 MHz by permission of I.C.I. (Polymer and Petrochemicals Division). O.r.d. and c.d. measurements were made with a Thorn–Bendix instrument. Paper chromatographic analysis (two-dimensional) was carried out at 20 ± 2° on Whatman no. 2 paper (27·5 × 27·5 cm²) in the solvent systems (A) 6% acetic acid (v/v) and (B) butan-2-ol–acetic acid–water (14 : 1 : 5 v/v). Procyanidins, (+)-catechin and (−)-epicatechin, were detected by illumination with u.v. light and by use of two spray reagents: (i) 2,6-dibromobenzoquinone 4-chloroimide (Gibbs reagent; 0·5% w/v in acetone) followed by sodium hydrogen carbonate (saturated aqueous solution) (spots appeared mauve-purple on a white background) and (ii) ferric chloride–potassium ferricyanide (freshly prepared 0·2% solutions containing a trace of potassium permanganate); successive washes with 2N-hydrochloric acid and finally water revealed readily oxidisable phenols as prussian blue spots on a white background. Chromatograms developed with system (ii) retained traces of hydrochloric acid and if placed in contact with undeveloped chromatograms (7 days at 20°) the procyanidins on the latter appeared as rose-pink brown spots and (+)-catechin and (−)-epicatechin as brown spots on a white background. Cyanidin was determined by measurement of its absorption at 535 nm and by chromatography on MN-300 cellulose-precoated plastic sheets with Forestal Solvent (R_F 0·49).[39]

Plant Survey.—Plant tissue (5—10 g) was macerated in a Waring Blendor with methanol (2 × 30 ml) and the methanolic solution, after removal of the plant debris, was extracted with light petroleum (b.p. 60—80°; 3 × 50 ml) and evaporated at 30° to *ca.* 10 ml. The solution was diluted with water (40 ml) and then extracted with ethyl acetate (8 × 30 ml). Evaporation of the combined ethyl acetate extracts at 30° gave the crude phenolic extract (*ca.* 0·1 g). A sample (0·01 g) was heated at 60° with ethanolic hydrochloric acid (5N; 2 ml) and after 20 min examined for the presence of cyanidin. The extract was analysed by paper chromatography and the presence of procyanidins was confirmed by co-chromatography with authentic samples. The results are described in Table 1 and Figure 1.

Isolation of Plant Procyanidins: General Method.—Plant material (1—2 kg) was treated as just described to give the crude phenolic extract (10—25 g). In some cases (*R.*

[49] T. A. Geissman and N. N. Yoshimura, *Tetrahedron Letters*, 1966, 2669.
[50] L. Jurd and R. Lundin, *Tetrahedron*, 1968, **24**, 2653.
[51] E. C. Bate-Smith and T. Swain, *Chem. and Ind.*, 1953, 377.
[52] D. E. Hathway and J. W. T. Seakins, *J. Chem. Soc.*, 1957, 1562; *Biochem. J.*, 1957, **67**, 239.
[53] B. R. Brown and R. J. Whiteoak, *J. Chem. Soc.*, 1964, 6084.

fructicosus, *R. idaeus*, *M. sylvestris*, *V. vitis-idaea*, and *S. caprea*) preliminary fractionation of the extract was achieved by chromatography (20 g of extract) on a Polyamide-Woelm column (60 × 3·5 cm) with methanol. Fractions (400 × 15 ml) were collected and analysed. Procyanidin-containing fractions (*ca.* 20—150) were combined to give a crude procyanidin mixture (*ca.* 5 g) which was further chromatographed on Sephadex LH-20 (60 × 2·5 cm) in ethanol or ethanol–propan-1-ol (1 : 1 v/v). Alternatively, in the case of extracts from *P. gratissima*, *T. cacao*, *Fragaria x anannasa*, and *A. hippocastanum*, the crude phenolic mixture was applied directly to Sephadex LH-20 columns. The eluate was fractionated (500 × 12 ml) and analysed by paper chromatography. The elution pattern from Sephadex LH-20 in ethanol followed the order (i) flavan-3-ols, (+)-catechin, (−)-epicatechin; (ii) procyanidin dimers B-2 ⟶ B-1 ⟶ A-1, A-2, B-5, B-8 ⟶ B-3, B-4 ⟶ B-6, B-7; and (iii) procyanidin oligomers (C-1, C-2, D-1, D-2). Appropriate fractions were combined and rechromatographed in the same system to yield procyanidins which ran as single compact spots (Figure 1). Final purification, before analysis, was achieved by filtration in acetone through a short column of Sephadex LH-20 (10 × 2·5 cm) and the products were isolated as off-white solids by evaporation or by freezing from t-butyl alcohol. This method sufficed for the isolation of procyanidins B-2, B-3, B-4, and B-8 from the sources indicated (Table 1).

Samples for analysis were dried at 75° and 0·1 mmHg for 48 h. Procyanidins kept in light slowly darkened but were regenerated by filtration in acetone through Sephadex LH-20.

Procyanidin B-2.—The *procyanidin* was isolated from the sources indicated (Table 1) (Found: C, 59·9; H, 5·0. $C_{30}H_{26}O_{12},H_2O$ requires C, 60·4; H, 4·7%), $[\alpha]_{578}^{20}$ + 15·2° (*c* 1·2 in EtOH); R_F(A) 0·58, R_F(B) 0·42. Deca-acetyl-B-2 was isolated as an amorphous solid after purification by t.l.c. [acetone–benzene (8 : 2 v/v); silica; R_F 0·50] (Found: C, 60·0; H, 4·9. Calc. for $C_{50}H_{46}O_{22}$: C, 60·1; H, 4·7%).

Octamethyl B-2 was prepared in methanol by use of an excess of diazomethane (3 × 24 h) and purified by t.l.c. [chloroform–methanol (500 : 1 v/v); silica; R_F 0·56] to give an amorphous solid (Found: C, 66·5; H, 5·7. $C_{38}H_{42}O_{12}$ requires C, 66·1; H, 6·1%), *m/e* 690 (M^+), 672, and 654.

Treatment of the procyanidin B-2 (0·5 mg) with 0·1N-hydrochloric acid in ethanol (2 ml) at 60° for 15 min gave (−)-epicatechin, which was detected by paper chromatography. Cyanidin (30%; $\lambda_{max.}$ 535 nm) was formed when the procyanidin was treated with 5N-hydrochloric acid in ethanol.

Procyanidin B-3.—The *procyanidin* was isolated from the sources outlined (Table 1) (Found: C, 60·5; H, 5·2. $C_{30}H_{26}O_{12},H_2O$ requires C, 60·4; H, 4·7%), $[\alpha]_{578}^{20}$ −244·7° (*c* 2·0 in EtOH); R_F(A) 0·43, R_F(B) 0·34; o.r.d. (*c* 0·93 in EtOH); $[\phi]_{250}$ −58,400, $[\phi]_{256}$ −37,280, $[\phi]_{263}$ −29,820, $[\phi]_{270}$ −21,740, $[\phi]_{278}$ −15,540, $[\phi]_{282}$ −13,600, $[\phi]_{286}$ −15,600, $[\phi]_{294}$ −13,660, $[\phi]_{310}$ −9200, $[\phi]_{330}$ −6900°; c.d. (*c* 0·69 in MeOH): $[\theta]_{244}$ −29,320, $[\theta]_{250}$ −1675, $[\theta]_{256}$ −526, $[\theta]_{263}$ −500; $[\theta]_{270}$ −530, $[\theta]_{278}$ −3350, $[\theta]_{286}$ −8375, $[\theta]_{296}$ −1540°.

The procyanidin when heated in acid gave (+)-catechin and cyanidin (25%).

Deca-acetyl-B-3 was isolated as an amorphous solid after purification by t.l.c. [acetone–benzene (8 : 2 v/v); silica; R_F 0·46] (Found: C, 59·7; H, 4·6. Calc. for $C_{50}H_{46}O_{22}$: C, 60·1; H, 4·7%).

Octamethyl-B-3 was prepared in methanol by use of an excess of diazomethane (3 × 24 h) and purified as before (R_F 0·52) to give an amorphous solid (Found: C, 66·6; H, 5·7. $C_{38}H_{42}O_{12}$ requires C, 66·1; H, 6·1%), M^+ 690.

Procyanidin-B-4.—The *procyanidin* was isolated from the sources outlined (Table 1) (Found: C, 59·9; H, 5·1. $C_{30}H_{26}O_{12},H_2O$ requires C, 60·4; H, 4·7%), $[\alpha]_{578}$ −193·5° (*c* 1·0 in EtOH); R_F(A) 0·50, R_F(B) 0·40; o.r.d. (*c* 0·8 in EtOH): $[\phi]_{250}$ −50,900, $[\phi]_{256}$ −34,900, $[\phi]_{263}$ −26,800, $[\phi]_{270}$ −21,800, $[\phi]_{278}$ −16,000, $[\phi]_{283}$ −12,500, $[\phi]_{286}$ −13,100, $[\phi]_{294}$ −12,300, $[\phi]_{312}$ −7300, $[\phi]_{333}$ −5800°; c.d. (*c* 0·55 in EtOH): $[\theta]_{244}$ −27,300, $[\theta]_{250}$ −6305, $[\theta]_{256}$ −536, $[\theta]_{263}$ −505, $[\theta]_{270}$ −626, $[\theta]_{278}$ −5266, $[\theta]_{286}$ −6305, $[\theta]_{294}$ −2102°.

Deca-acetyl-B-4 crystallised from ethanol as needles, m.p. 171—172° (lit.,[15] 172—173°) (Found: C, 60·1; H, 4·8. Calc. for $C_{30}H_{46}O_{22}$: C, 60·1; H, 4·7%) M^+, 998; $[\alpha]_{578}$ −130·8° (*c* 2·0 in $CHCl_3$); R_F 0·42 [acetone–benzene (8 : 2 v/v); silica]; o.r.d. (*c* 1·07 in $CHCl_3$): $[\phi]_{256}$ −18,780, $[\phi]_{263}$ −10,260, $[\phi]_{270}$ −13,520, $[\phi]_{278}$ −24,710, $[\phi]_{286}$ −29,840, $[\phi]_{294}$ −19,580, $[\phi]_{303}$ −14,920, $[\phi]_{312}$ −12,130, $[\phi]_{323}$ −10,730, $[\phi]_{333}$ −8860, $[\phi]_{400}$ −4660°.

Octamethyl-B-4 was prepared in methanol by use of an excess of diazomethane (3 × 24 h) and purified as before (R_F 0·50) to give an amorphous solid (Found: C, 66·5; H, 5·8. $C_{38}H_{42}O_{12}$ requires C, 66·1; H, 6·1%), M^+, 690.

Procyanidin B-4, when heated in acid, gave (−)-epicatechin and cyanidin (25%).

Procyanidin B-8.—The *procyanidin* was isolated from *R. fructicosus* and *R. idaeus* (Found: C, 59·9; H, 5·1. $C_{30}H_{26}O_{12},H_2O$ requires C, 60·4; H, 4·7%), $[\alpha]_{578}^{20}$ −160·3° (*c* 2·3 in MeOH); R_F(A) 0·50, R_F(B) 0·52; o.r.d. (*c* 2·3 in MeOH): $[\phi]_{400}$ −3056, $[\phi]_{345}$ −4850, $[\phi]_{303}$ −8403, $[\phi]_{294}$ −9930, $[\phi]_{286}$ −12,200, $[\phi]_{278}$ −9950, $[\phi]_{270}$ −16,810, $[\phi]_{263}$ −24,030, $[\phi]_{256}$ −28,590, $[\phi]_{250}$ −40,240°; c.d. (*c* 2·3 in MeOH): $[\theta]_{303}$ −510, $[\theta]_{294}$ −890, $[\theta]_{286}$ −2040, $[\theta]_{278}$ −1400, $[\theta]_{270}$ −500, $[\theta]_{263}$ −1010, $[\theta]_{256}$ −3060, $[\theta]_{250}$ −6120°. Methylation (×3) of a sample (0·01 g) with diazomethane in methanol and separation of the product by t.l.c. [silica; chloroform–methanol (0·5% v/v)] gave an amorphous solid which gave a molecular ion at *m/e* 690. Treatment of the procyanidin with acid gave (−)-epicatechin and cyanidin (30%). The reaction of the procyanidin (0·005 g) with toluene-α-thiol (0·6 ml) and acetic acid (0·3 ml) in ethanol (2 ml) and analysis (24 and 36 h) by paper chromatography showed the formation of (−)-epicatechin and the benzylthioflavans (XIII) and (XIV). After 36 h the reaction mixture was stirred with Raney nickel (3 ml ethanolic slurry) for 2 h at room temp.; paper chromatography then showed (−)-epicatechin and (+)-catechin to be present.

Isolation of Procyanidins from Aesculus hippocastanum.—Freshly collected fruit (September) were peeled and the shells (750 g) extracted as before to give the crude extract (15 g), which was chromatographed directly on Sephadex LH-20 (60 × 3·5 cm column) in ethanol. Elution with ethanol gave fractions (300 × 12 ml) which were grouped as shown. Group 1 (25—50) gave (−)-epicatechin (3·0 g) after two crystallisations from water; m.p. and mixed m.p. 240—242° (lit.,[54] 242°) (Found: C, 61·9; H, 5·0. Calc. for $C_{15}H_{14}O_6$: C, 62·0; H, 4·8%), $[\alpha]_{578}$ −57·6° (*c* 2·1 in Me_2CO);

[54] Heilbron's 'Dictionary of Organic Compounds,' vol. 1, Eyre and Spottiswoode, London, 1965, p. 572.

R_F(A) 0·37, R_F(B) 0·50; o.r.d. (*c* 0·85 in EtOH): $[\phi]_{250}$ −938, $[\phi]_{256}$ −940, $[\phi]_{263}$ −1025, $[\phi]_{270}$ −1700, $[\phi]_{278}$ 2220, $[\phi]_{282}$ −2270, $[\phi]_{286}$ −1970, $[\phi]_{294}$ −1190, $[\phi]_{303}$ −768, $[\phi]_{333}$ −427, $[\phi]_{400}$ −170°; c.d. (*c* 1·08 in MeOH): $[\theta]_{256}$ −90, $[\theta]_{263}$ −537, $[\theta]_{270}$ −2015, $[\theta]_{278}$ −4030, $[\theta]_{286}$ −2416, $[\theta]_{294}$ −671, $[\theta]_{303}$ 0°. Acetylation gave (−)-epicatechin penta-acetate, m.p. and mixed m.p. 151—152°, $[\alpha]_{578}$ −17·0° (*c* 1·8 in $CHCl_3$); o.r.d. (*c* 1·65 in $CHCl_3$): $[\phi]_{250}$ +3480, $[\phi]_{256}$ +2880, $[\phi]_{263}$ +2420, $[\phi]_{270}$ 0, $[\phi]_{278}$ −3410, $[\phi]_{286}$ −3400, $[\phi]_{294}$ −1660, $[\phi]_{303}$ −1130, $[\phi]_{323}$ −680°; c.d. (*c* 1·65 in $CHCl_3$): $[\theta]_{250}$ 0, $[\theta]_{256}$ −2420, $[\theta]_{263}$ −4850, $[\theta]_{270}$ −6670, $[\theta]_{278}$ −4550°.

Methylation with diazomethane gave tetramethyl-epicatechin, m.p. and mixed m.p. 153—154° (Found: C, 65·9; H, 6·8. Calc. for $C_{19}H_{22}O_6$: C, 65·9; H, 6·7%), $M^{\bullet+}$, 346; $[\alpha]_{578}$ −62° (*c* 2·1 in $CHCl_3$).

Group 2 (51—70) gave, after further chromatography on Sephadex LH-20, procyanidin B-2 (2·3 g).

Group 3 (71—100), on paper chromatographic analysis, showed the presence of procyanidins A-2, B-1, and B-5 (Figure 1). Trituration with water gave procyanidin A-2 as needles (0·95 g), m.p. >280° (Found: C, 58·6; H, 4·8. Calc. for $C_{30}H_{24}O_{12},2H_2O$: C, 58·8; H, 4·6%), $[\alpha]_{578}$ +56·9° (*c* 0·62 in MeOH); R_F(A) 0·24, R_F(B) 0·51. The nona-acetate of A-2 had m.p. 156—157° (lit.,[17] 158—159°); $[\alpha]_{578}$ −88·6°.

Group 4 (101—130), on paper chromatographic analysis showed the presence of A-1, A-2, B-1, B-5, and C-1 (Figure 1) and procyanidin A-2 (0·35 g) was isolated as before.

The mother liquors from groups 3 and 4 were combined and evaporated to a gum at 30°. The latter was dissolved in methanol and streaked on plates (25 cm^2) of microgranular cellulose CT (Reeve–Angel), which were developed in solvent system (A). Bands (R_F *ca.* 0·55—0·65 and 0·4—0·5) were separated and the components reisolated with methanol to give a mixture of procyanidins B-1 and C-1 (*ca.* 0·35 g) and crude B-5 (*ca.* 0·1 g). Rechromatography on cellulose plates of each fraction in solvent system (B) gave the individual procyanidins, which were finally purified by chromatography in acetone on Sephadex LH-20.

Procyanidin B-1 was obtained as an off-white amorphous solid (0·12 g) (Found: C, 60·0; H, 5·2. $C_{30}H_{26}O_{12},H_2O$ requires C, 60·4; H, 4·7%), $[\alpha]_{578}$ +31° (*c* 0·8 in EtOH); R_F(A) 0·51, R_F(B) 0·30.

Deca-acetyl-B-1 was prepared and purified as before to give rosettes, m.p. 228—229° (lit.,[15] 231—232°); $[\alpha]_{578}$ +110°; R_F 0·53 [acetone–benzene (8 : 2 v/v); silica]; o.r.d. (*c* 1·21 in $CHCl_3$): $[\phi]_{250}$ +49,500, $[\phi]_{256}$ +26,400, $[\phi]_{263}$ +16,500, $[\phi]_{270}$ +11,960, $[\phi]_{278}$ +11,200, $[\phi]_{286}$ +14,850, $[\phi]_{294}$ +9890, $[\phi]_{303}$ +7830, $[\phi]_{312}$ +6600, $[\phi]_{333}$ +4950°; c.d. (*c* 1·21 in $CHCl_3$): $[\theta]_{250}$ +9890, $[\theta]_{256}$ +3120, $[\theta]_{263}$ +3300, $[\theta]_{270}$ +5770, $[\theta]_{278}$ +8250, $[\theta]_{286}$ +7000, $[\theta]_{294}$ +2060, $[\theta]_{303}$ +1650°.

Octamethyl-B-1 was obtained by treatment with diazomethane in methanol as before (Found: C, 66·4; H, 5·7. $C_{38}H_{42}O_{12}$ requires C, 66·1; H, 6·1%), M^+, 690.

Procyanidin B-1, when heated in acid, gave (+)-catechin and cyanidin (30%).

Procyanidin B-5 was isolated as an off-white amorphous solid (0·03 g) (Found: C, 59·9; H, 5·0. $C_{30}H_{26}O_{12},H_2O$ requires C, 60·4; H, 4·7%), $[\alpha]_{578}$ +119·2° (*c* 1·35 in MeOH); R_F(A) 0·38, R_F(B) 0·43. Methylation (3 ×) of a sample (0·01 g) with diazomethane in methanol and separation of the product by t.l.c. [silica; chloroform–methanol (0·5% v/v)] gave an amorphous solid, $M^{\bullet+}$, 690.

Treatment of the procyanidin with acid gave (−)-epicatechin and cyanidin (30%). The reaction of the procyanidin (0·005 g) with toluenethiol (0·6 ml) and acetic acid (0·3 ml) in ethanol (2 ml) [analysis (24 and 36 h) by paper chromatography] gave (−)-epicatechin and the benzylthioflavan (XI). After 36 h the reaction mixture was stirred with Raney nickel (3 ml ethanolic slurry) for 2 h at room temperature; paper chromatographic analysis then showed only (−)-epicatechin to be present.

Procyanidin C-1 was isolated as a buff-coloured amorphous solid (0·01 g) (Found: C, 59·5; H, 4·9. $C_{45}H_{38}O_{18},2H_2O$ requires C, 59·9; H, 4·7%); R_F(A) 0·48, R_F(B) 0·28. Methylation of the procyanidin (0·005 g) with diazomethane in methanol (3 ×) and purification by t.l.c. [methanol–chloroform (0·5% v/v); R_F 0·4] gave the methyl ether as a gum, $M^{\bullet+}$, 1034. Treatment of the procyanidin (1 mg) in 0·1N-hydrochloric acid in ethanol (2 ml) at room temperature gave (−)-epicatechin after 36 h. After 1, 3, 5, and 8 h procyanidin B-2 was detected in addition to (−)-epicatechin.

The procyanidin (5 mg) was treated with toluenethiol (0·6 ml) and acetic acid (0·3 ml) in ethanol (2 ml) (see later); analysis (8, 24, and 36 h) by paper chromatography showed the presence of (−)-epicatechin, procyanidin B-2, the benzylthioflavan (XI) and a compound [R_F(A) 0·54, R_F(B) 0·71] tentatively identified as (XVIII). After 36 h treatment (2 h) with Raney nickel (3 ml ethanolic slurry) showed only (−)-epicatechin to be present.

Treatment of the procyanidin with 5N-hydrochloric acid in ethanol at 60° gave cyanidin.

Isolation of Procyanidins from Vaccinium vitis-idaea.—Unripe berries (July—August) (600 g) gave a phenolic extract (11·5 g) which was initially fractionated on a Polyamide-Woelm column (30 × 2·5 cm) in methanol. The procyanidins were further fractionated on Sephadex LH-20 in ethanol (15 ml fractions). Tubes 0—75 gave (+)-catechin, (−)-epicatechin, procyanidin B-2 (10 mg), procyanidin B-3 (15 mg), procyanidin B-5 (15 mg), and procyanidin B-1 (25 mg) as before. Material from tubes 79—98 and 99—116 was triturated with water and gave procyanidin A-2 (0·12 g). The mother liquor from the latter fraction was combined with the contents of tubes 117—136 and rechromatographed in ethanol–propan-1-ol (4 : 1 v/v) on Sephadex LH-20 (30 × 2·5 cm). Tubes 195—210 gave *procyanidin B*-7 (8 mg) as an off-white amorphous powder (Found: C, 59·9; H, 5·1. $C_{30}H_{26}O_{12},H_2O$ requires C, 60·4; H, 4·7%); R_F(A) 0·43, R_F(B) 0·29. The methyl ether (made with diazomethane in methanol), isolated as before, showed $M^{\bullet+}$, 690. Treatment of the procyanidin (3 mg) with toluenethiol (0·75 ml) and acetic acid (0·4 ml) in ethanol (2 ml) (see later) gave after 36 h the benzylthioflavan (XI) and (+)-catechin. Then the reaction mixture was treated with Raney nickel (3 ml ethanolic slurry) (2 h) and analysed by paper chromatography to show the presence of (+)-catechin and (−)-epicatechin.

Isolation of Procyanidins from Salix caprea.—Fresh male catkins (May) (600 g) gave a crude phenolic extract (10 g) which was applied directly to Sephadex LH-20 (40 × 3·5 cm) in ethanol. Fractions 18—28 gave (+)-catechin (0·8 g) as needles (from methanol), m.p. and mixed m.p. 177° (Found: C, 62·1; H, 5·1. Calc. for $C_{15}H_{14}O_6$: C, 62·1; H, 4·8%), $[\alpha]_{578}$ +17·8° (*c* 2·0 in EtOH); R_F(A) 0·47, R_F(B) 0·51; o.r.d. (*c* 1·56 in EtOH): $[\phi]_{250}$ +790, $[\phi]_{256}$ +744, $[\phi]_{263}$ +418, $[\phi]_{270}$ +279, $[\phi]_{278}$ −279, $[\phi]_{286}$ −604, $[\phi]_{294}$ −372, $[\phi]_{303}$ −93, $[\phi]_{312}$ +111, $[\phi]_{323}$ +186, $[\phi]_{333}$ +205°;

c.d. (*c* 0·85 in EtOH): $[\theta]_{250}$ −1364, $[\theta]_{256}$ −1705, $[\theta]_{263}$ −3700, $[\theta]_{270}$ −4094, $[\theta]_{278}$ −4070, $[\theta]_{286}$ −3400, $[\theta]_{294}$ −1024°.

Tetramethylcatechin, prepared with diazomethane, had m.p. and mixed m.p. 144—145° (Found: C, 65·8; H, 6·7. Calc. for $C_{19}H_{22}O_6$: C, 65·9; H, 6·7%), $[\alpha]_{578}$ −14·3° (*c* 1·5 in $CHCl_3$), M^+, 346.

(+)-Penta-acetylcatechin had m.p. and mixed m.p. 131—132° (Found: C, 60·2; H, 4·9. Calc. for $C_{25}H_{24}O_{11}$: C, 60·0; H, 4·8%), $[\alpha]_{578}$ +39·7°; o.r.d. (*c* 1·54 in $CHCl_3$): $[\phi]_{250}$ +7790, $[\phi]_{256}$ +6820, $[\phi]_{263}$ +6170, $[\phi]_{270}$ +3900, $[\phi]_{278}$ −974, $[\phi]_{286}$ −2920, $[\phi]_{294}$ −812, $[\phi]_{303}$ −487, $[\phi]_{312}$ −162°; c.d. (*c* 1·54 in $CHCl_3$): $[\theta]_{250}$ 0, $[\theta]_{256}$ −2920, $[\theta]_{263}$ −5520, $[\theta]_{270}$ −7790, $[\theta]_{278}$ −7780, $[\theta]_{286}$ −320°. Fractions 45—65 gave, after rechromatography on Sephadex LH-20, procyanidin B-3 (0·8 g).

Fractions 66—85 gave, after rechromatography on Sephadex LH-20 in ethanol, *procyanidin B*-6 (15 mg) as an off-white powder (Found: C, 60·0; H, 5·2. $C_{30}H_{26}O_{12},H_2O$ requires C, 60·4; H, 4·7%), $[\alpha]_{578}$ −130° (*c* 0·5 in EtOH); R_F(A) 0·46, R_F(B) 0·52; o.r.d. (*c* 0·5 in EtOH): $[\phi]_{250}$ −27,100, $[\phi]_{256}$ −22,200, $[\phi]_{263}$ −17,300, $[\phi]_{270}$ −13,600, $[\phi]_{278}$ −8630, $[\phi]_{286}$ −6170, $[\phi]_{294}$ −6200, $[\phi]_{303}$ −4900°. Methylation of the procyanidin (5 mg) with diazomethane (3 ×) in methanol gave, after purification by t.l.c. [methanol–chloroform (0·5% v/v); silica], a methyl ether, $M^{\cdot+}$, 690. Procyanidin B-6 (3 mg) was refluxed in a nitrogen stream in ethanol (2·5 ml) containing toluenethiol (0·8 ml) and acetic acid (0·5 ml). Paper chromatographic analysis showed after 36 h (+)-catechin and the benzylthioflavans (XIII) and (XIV). Treatment of the reaction mixture with Raney nickel (3 ml ethanolic slurry) (2 h) and subsequent paper chromatographic analysis showed only (+)-catechin. Fractions 86—120 gave, after rechromatography on Sephadex LH-20 in ethanol–propan-1-ol (4 : 1 v/v), further procyanidin B-6 (5 mg) and *procyanidin C*-2, which was finally isolated by t.l.c. on microgranular cellulose [solvent system (B)] as a white amorphous solid (16 mg) (Found: C, 59·6; H, 4·6. $C_{45}H_{38}O_{18},2H_2O$ requires C, 59·9; H, 4·7%), $[\alpha]_{578}$ −173° (*c* 1·03 in EtOH); R_F(A) 0·40, R_F(B) 0·29; o.r.d. (*c* 1·03 in EtOH): $[\phi]_{250}$ −10,680, $[\phi]_{256}$ −7740, $[\phi]_{263}$ −6640, $[\phi]_{270}$ −5520, $[\phi]_{278}$ −4040, $[\phi]_{286}$ −3320, $[\phi]_{294}$ −3320, $[\phi]_{303}$ −2580, $[\phi]_{312}$ −2400, $[\phi]_{323}$ −1840°. Methylation of the procyanidin (5 mg) with diazomethane (3 ×) in methanol gave, after purification by t.l.c. [methanol–chloroform (0·5% v/v); silica gel], a methyl ether, M^+ 1034. Procyanidin C-2 (5 mg) was heated under reflux in ethanol (2·5 ml) containing toluenethiol (0·2 ml) and acetic acid (0·5 ml). Paper chromatographic analysis showed, after 8 h, (+)-catechin, procyanidin B-3, the benzylthioflavans (XIII) and (XIV), and a compound, R_F(A) 0·41, R_F(B) 0·59, tentatively formulated as (XIX). After 36 h only (+)-catechin and the thioethers (XIII) and (XIV) were detected. Procyanidin C-2 (1 mg) was treated with 0·1N-hydrochloric acid in ethanol (1·5 ml) at room temperature; by paper chromatography (1, 8, 24, and 48 h) detected (+)-catechin and procyanidin B-3 after 1, 8, and 24 h.

(2R,3S,4S)-4-*Benzylthioflavan*-3,3′,4′,5,7-*pentaol* (XI).—Toluene-α-thiol (2 ml) and acetic acid (1 ml) were added to an ethanolic solution (4 ml) of procyanidin B-2 (0·27 g) and the mixture was refluxed under nitrogen for 24 h. Removal of the solvents at 30° left an oily residue which was dissolved in chloroform–propan-1-ol (4 : 1 v/v) and chromatographed on a column of Sephadex LH-20 (30 × 1·5 cm) (200 × 10 ml fractions). Fractions 142—174 gave (−)-epicatechin (0·12 g, 88%), m.p. and mixed m.p. 241—243°. Fractions 82—102 gave, after freeze-drying from t-butyl alcohol, the thioether as a white amorphous solid (0·15 g, 75%), $[\alpha]_{578}$ −21·1° (*c* 1·2 in EtOH), R_F(A) 0·35, R_F(B) 0·79. Treatment of the thioether (2 mg) with 5N-hydrochloric acid in ethanol (4 ml) at 60° gave cyanidin (50%). Treatment of the thioether (2 mg) in ethanol (5 ml) with Raney nickel (3 ml ethanolic slurry) for 2 h gave (−)-epicatechin, which was detected by paper chromatography.

The *tetramethyl ether* was prepared with diazomethane in methanol (3 ×) and purified by t.l.c. (silica; chloroform); it crystallised from methanol–water as needles, m.p. 57—58° (Found: C, 66·4; H, 5·8; S, 6·7. $C_{26}H_{28}O_6S$ requires C, 66·7; H, 6·0; S, 6·8%), $M^{\cdot+}$, 468, $[\alpha]_{578}$ −10·8° (*c* 0·8 in $CHCl_3$).

The *penta-acetate* was prepared in pyridine–acetic anhydride and purified by t.l.c. [methanol–chloroform (0·5% v/v); silica]; it crystallised from methanol as needles, m.p. 125—126° (Found: C, 61·4; H, 5·1; S, 5·2. $C_{32}H_{30}O_{11}S$ requires C, 61·7; H, 4·8; S, 5·1%), $M^{\cdot+}$, 622, $[\alpha]_{578}$ −35·3° (*c* 0·7 in $CHCl_3$).

(2R,3R,4S)-4-*Benzylthioflavan*-3,3′,4′,5,7-*pentaol* (XIV).—Procyanidin B-4 (0·53 g) was refluxed in ethanol (4 ml) containing toluenethiol (2 ml) and acetic acid (1 ml) under a nitrogen stream for 24 h. The solvents were removed at 30° and the oily residue was chromatographed in chloroform–propan-1-ol (4 : 1 v/v) on Sephadex LH-20 (30 × 1·5 cm). Fractions 270—305 gave (−)-epicatechin (0·22 g, 83%), m.p. and mixed m.p. 241—242°. The thioether (0·20 g, 53%) was obtained as a white amorphous powder from fractions 154—194 after freeze-drying from t-butyl alcohol; $[\alpha]_{578}$ +45·1° (*c* 0·8 in EtOH); R_F(A) 0·45, R_F(B) 0·80.

The *tetramethyl ether*, prepared with diazomethane, crystallised as needles from ethyl acetate–methanol, m.p. 127—128° (Found: C, 66·5; H, 6·2; S, 7·0%; $M^{\cdot+}$, 468·1594. $C_{26}H_{28}O_6S$ requires C, 66·7; H, 6·0; S, 6·8%; *M*, 468·1607), $[\alpha]_{578}$ +148° (*c* 1·3 in $CHCl_3$).

The *penta-acetate*, prepared with pyridine–acetic anhydride, crystallised from methanol as needles, m.p. 69° (Found: C, 61·9; H, 4·9; S, 5·1. $C_{32}H_{30}O_{11}S$ requires C, 61·7; H, 4·8; S, 5·1%), $M^{\cdot+}$, 622, $[\alpha]_{578}$ +79·1° (*c* 1·2 in $CHCl_3$).

Fractions 126—146, analysed by paper chromatography, contained the thioethers (XIII) and (XIV). Rechromatography gave a compound (0·014 g) formulated as (XIV) (^{1}H n.m.r. shown in Table 4).

Treatment of both thioethers (XIII) and (XIV) with acid (5N-hydrochloric acid in ethanol) gave cyanidin. Treatment with Raney nickel (2 h) gave (+)-catechin in both cases.

Analytical Degradation of Procyanidins with Toluene-α-thiol.—The procyanidin (2—5 mg) in ethanol (2 ml), toluenethiol (0·75 ml), and acetic acid (0·4 ml) was refluxed under nitrogen. Samples were withdrawn after 0·5, 1, 2, 8, and 24 h and analysed by paper chromatography [solvent systems (A) and (B)]. Raney nickel (3 ml ethanolic slurry) was added to the mixture and the solution stirred for 2 h at room temp. The catalyst was filtered off and the solution again analysed by paper chromatography.

[*u*-^{14}C]-(−)-*Epicatechin*.—Fresh shoots (*ca.* 3 g; *Crataegus monogyna* or *Rubus idaeus*) were fed [*u*-^{14}C]glucose (0·067 mCi, 2·1 × 10^{-3} mmol) by immersing the ends of the cut shoots in vials containing a solution (5 ml) of the radioactive

substrate. The vials were placed in a slight draught and replenished with water when the radioactive solution was absorbed. After 7 days the plant material was extracted as before and (−)-epicatechin was isolated after chromatography on Sephadex LH-20. Unlabelled (−)-epicatechin (15 mg) was added and the product was recrystallised to constant activity (10 mg; total activity 48,200 decomp. min^{-1}).

The ^{14}C-labelled (−)-epicatechin was administered in the same way to cut shoots of *R. idaeus* (2 g fresh weight) and after 7 days (−)-epicatechin and the procyanidin B-4 were isolated. Unlabelled (−)-epicatechin (15 mg) was added to the former sample and the whole was recrystallised to constant activity (8 mg; total activity 11,100 decomp. min^{-1}). The procyanidin was converted into its deca-acetate, diluted with unlabelled material (15 mg), and crystallised to constant activity (12 mg; total activity 6700 decomp. min^{-1}).

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