

Plant Proanthocyanidins. Part 3.¹ Conformational and Configurational Studies of Natural Procyanidins

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Means to generate the C-4 carbocations (3) and (4) corresponding to (+)-catechin (1) and (-)-epicatechin (2), respectively, are outlined, and the use of these intermediates for the synthesis of model procyanidins and for the biogenetically patterned synthesis of natural procyanidins is discussed. ¹³C N.m.r. data for model flavan systems and natural procyanidins are reported and analysed and the information is used to assign the 4*R*-configuration to four natural procyanidin dimers. The phenomenon of conformational isomerism is demonstrated for the natural procyanidin dimers, and two different forms of restricted rotation about the interflavan bond are proposed. The information is used to clarify many earlier structural anomalies, to predict preferred conformations, and to specify a C(4)–C(8) link for the four principal dimers (B-1—4). The properties of some procyanidin polymers are noted, and structures of opposite helicity are proposed for two of the major types found in nature.

MANY plants, particularly those with a woody habit of growth, contain colourless phenolic substances (proanthocyanidins) which release anthocyanidins when

¹ Part 2, D. Jacques, E. Haslam, D. Greatbanks, and G. R. Bedford, *J.C.S. Perkin I*, 1974, 2663.

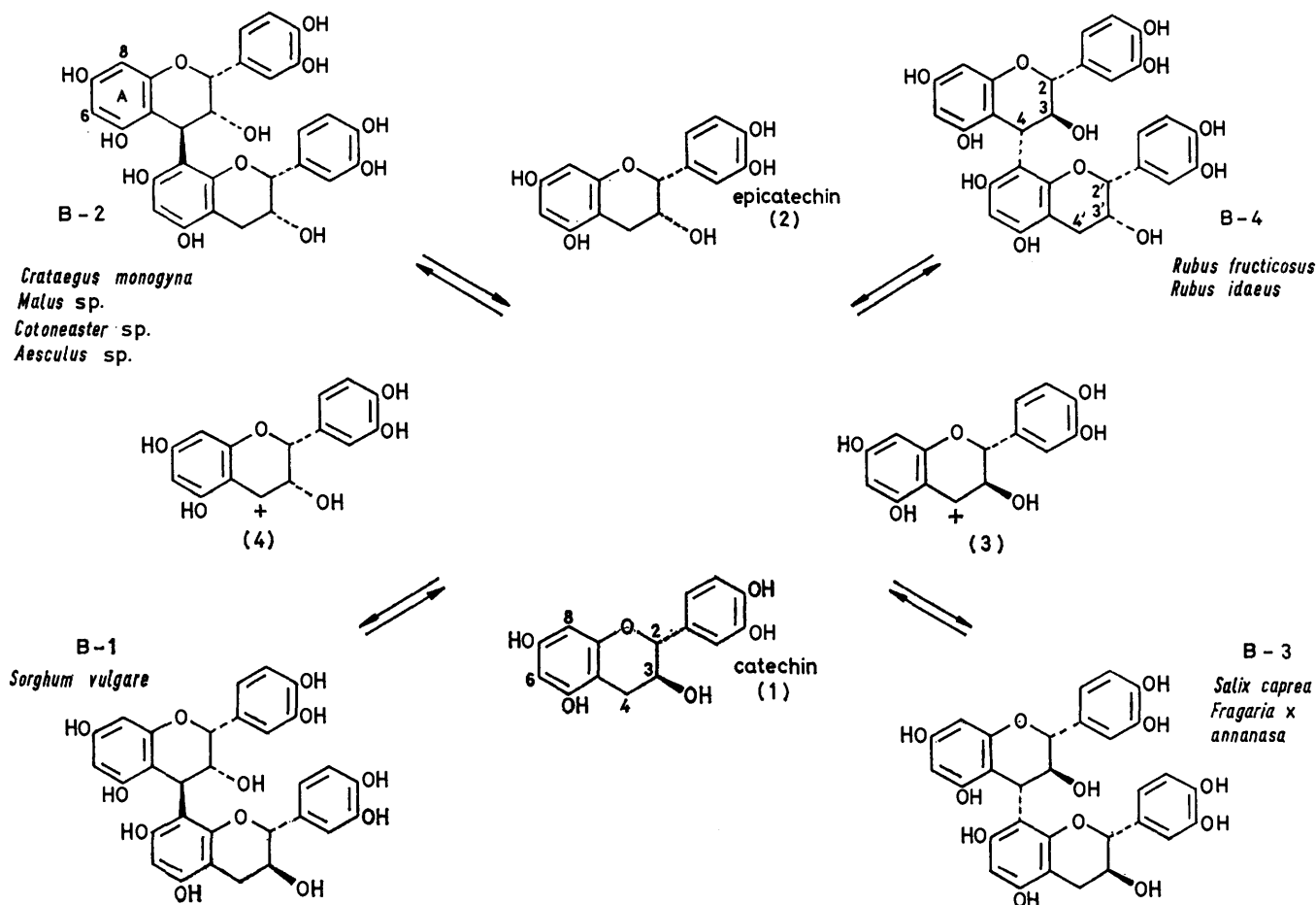
treated with acid and may be astringent to the taste.² The most widely distributed of these proanthocyanidins in nature are the dimers and higher oligomers of the

² E. Haslam, 'The Flavonoids,' ed. T. J. Mabry, H. Mabry, and J. B. Harborne, Chapman and Hall, 1975, p. 505.

procyanidin B group, which biosynthetic studies have shown^{3,4} are metabolised by reaction between (+)-catechin (1) or (-)-epicatechin (2) and the related carbocations (3) and (4) (Scheme). Each of the four biosynthetic reactions yields one predominant procyanidin dimer (B-1—4, respectively) and these particular compounds have been the subject of a number of studies.^{5,6} These investigations however left several critical problems unanswered. Thus although a synthesis of procyanidin B-3 octamethyl ether diacetate⁷ established the

outlined here and some comment is also made on the factors which govern the preference for the formation of the four procyanidins (B-1—4) in each of the biosynthetic reactions and thus determine that they predominate in nature. Some preliminary details of this work have been published.^{4,9}

Structural analysis of the procyanidins has been aided by the preparation of a series of a model compounds (5)—(15). Reaction of flavan-4 α -ol with resorcinol¹⁰ or phloroglucinol in acidic ethand gave, after acetyla-



SCHEME Biogenetically patterned procyanidin synthesis

position of the interflavan link in this dimer, its position in the remaining procyanidins was not determined. Similarly, whilst hindered rotation about the interflavan bond had been advanced^{5,8} as an explanation for the anomalous ¹H n.m.r. spectra displayed by the procyanidins, no firm proof of the existence of this phenomenon had been advanced. Solutions to these problems are

³ D. Jacques and E. Haslam, *J.C.S. Chem. Comm.*, 1974, 231.

⁴ E. Haslam, *J.C.S. Chem. Comm.*, 1974, 594.

⁵ R. S. Thompson, D. Jacques, E. Haslam, and R. J. N. Tanner, *J.C.S. Perkin I*, 1972, 1387.

⁶ K. Weinges, W. Kaltenhauser, H.-D. Marx, E. Nader, F. Nader, J. Perner, and D. Seiler, *Annalen*, 1968, 711, 184.

⁷ K. Weinges, H.-D. Marx, and J. Perner, *Chem. Ber.*, 1970, 103, 2344.

tion, the 4-arylflavan derivatives (5)—(7). The compounds are formulated (one stereoisomeric form depicted) on the basis of ¹H n.m.r. analysis ($J_{3ax,4} 3.8$, $J_{3eq,4} 4.0$ Hz) as the 2,4-*trans*-isomers. Analogous model systems where the flavan unit is related to (+)-catechin (1) and (-)-epicatechin (2) were prepared by treating the polymeric procyanidins from, respectively, *Salix caprea* catkins and *Butea frondosa* gum with phloroglucinol or

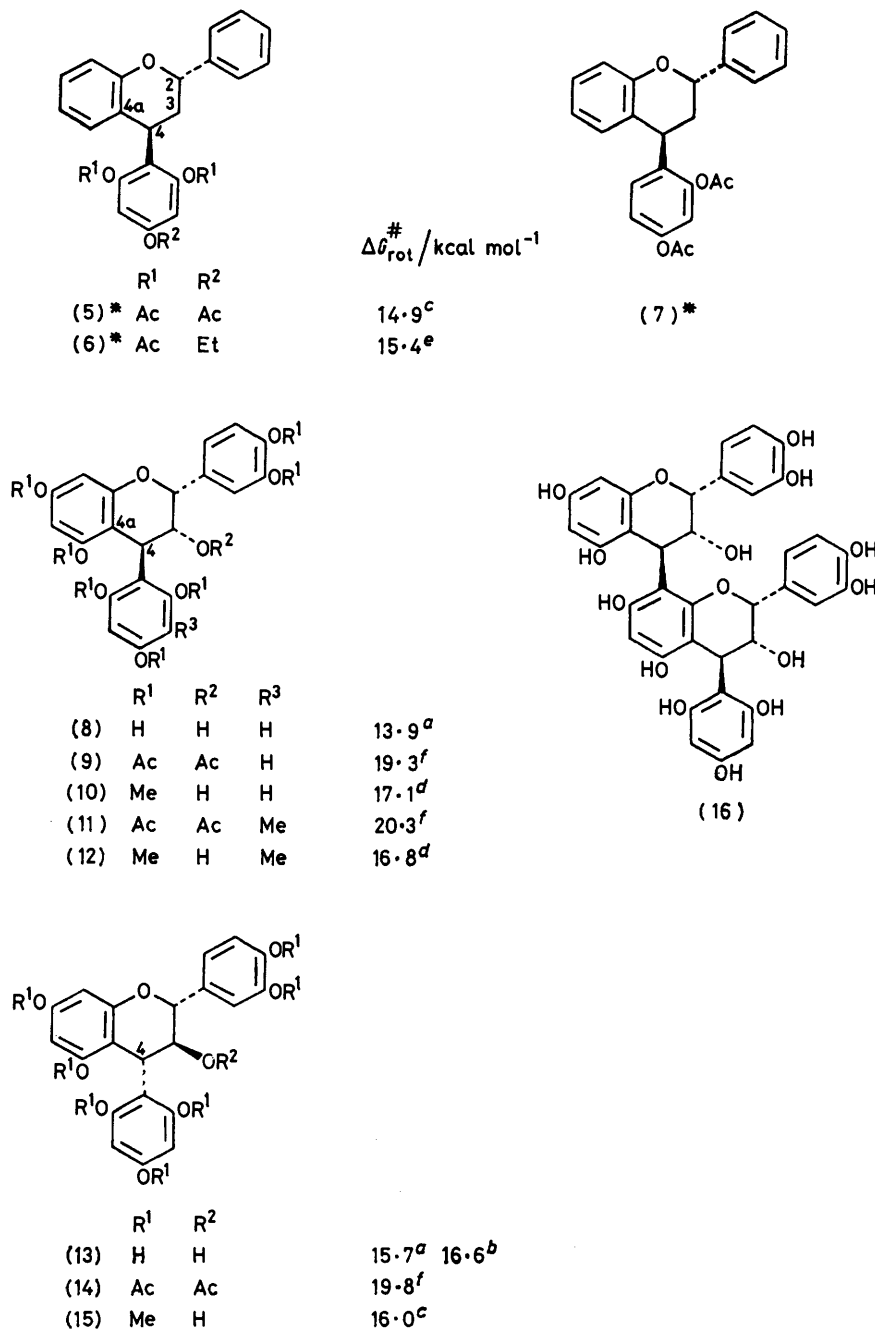
⁸ K. Weinges, H.-D. Marx, and K. Göritz, *Chem. Ber.*, 1970, 103, 2336.

⁹ A. C. Fletcher, L. J. Porter, and E. Haslam, *J.C.S. Chem. Comm.*, 1976, 627.

¹⁰ B. R. Brown, H. Cummings, and J. Newbould, *J. Chem. Soc.*, 1961, 3677.

2,4,6-trihydroxytoluene. The procyanidin from *Butea frondosa* was first described by Robinson,¹¹ and although Seshadri and Ganguly¹² later attributed a flavan-3,4-

(13)—(15) was based on the ¹H 3,4-coupling constant (10.0 Hz), but the small value (2—3 Hz) of the analogous coupling constant in the series (8)—(12)—as in the



^a [²H₆]Acetone.
^f Nitro[²H₆]benzene.

^b [²H₆]Dimethyl sulphoxide.

^c [²H₅]Pyridine.

^d [²H₅]Pyridine—[²H]chloroform.

^e [²H]Chloroform

* Racemate.

diol structure to it the physical properties (*e.g.* solubility and immobility on paper chromatographic analysis) indicate that this designation is incorrect. The assignment of absolute stereochemistry at C-4 in compounds

natural procyanidins B-1 and B-2^{5,6}—did not permit an unequivocal determination of stereochemistry for these compounds. A minor product isolated in the reaction of phloroglucinol and (4) was the phenol (16).

¹¹ G. M. Robinson, *J. Chem. Soc.*, 1937, 1157.

¹² A. K. Ganguly and T. R. Seshadri, *Tetrahedron*, 1959, **6**, 21.

Determination of the absolute stereochemistry of the models (8)—(12) and the procyanidin dimers B-1, -2, -5,

TABLE 1

^{13}C N.m.r. chemical shifts of flavan and substituted flavan derivatives (δ values; Me_4Si standard; solvent [^2H]-chloroform unless otherwise stated)

Flavan derivatives	C-2	C-3	C-4
Flavan (17)	77.6	29.9	25.0
-4-ol (2,4- <i>cis</i>) (19)	77.0	40.2	65.8
-4-ol acetate (2,4- <i>cis</i>)	76.3	35.6	67.4
-4-ol (2,4- <i>trans</i>) (18)	73.0	38.3	63.9
-4-ol acetate (2,4- <i>trans</i>)	73.5	35.9	65.7
3',4',5,7-Tetramethoxy (25)	77.8	29.6	19.5
4-(2,4,6-Triacetoxyphenyl) (2,4- <i>trans</i>) (5)	74.9	32.9	29.0
4-(2,6-Diacetoxy-4-ethoxyphenyl) (2,4- <i>trans</i>) (6)	75.0	33.1	28.8
4-phenyl (2,4- <i>cis</i>)	78.0	40.3	43.4
4-phenyl (2,4- <i>trans</i>)	73.1	39.2	40.2
(+) -Catechin derivatives			
(+) -Catechin (1) ($[\text{C}_6\text{H}_6]$ acetone)	82.3	68.2	28.4
(+) -Catechin (1) ($[\text{C}_6\text{H}_6]$ DMSO)	80.7	66.1	27.6
3',4',5,7-Tetramethyl- * (20)	81.7	68.1	27.8
3-Acetyl-3',4',5,7-tetramethyl- *	78.5	69.2	24.0
3,3',4',5,7-Penta-acetyl- *	78.6	68.2	23.9
(2 <i>R</i> ,3 <i>S</i> ,4 <i>S</i>)-3',4',5,7-Tetramethoxyflavan-3,4-diol (22)	80.7	73.8	70.4
(2 <i>R</i> ,3 <i>S</i> ,4 <i>R</i>)-3',4',5,7-Tetramethoxyflavan-3,4-diol * (21)	76.7	70.6	61.5
(2 <i>R</i> ,3 <i>S</i> ,4 <i>S</i>)-4-(2,4'',6''-Trihydroxyphenyl)flavan-3,3',4',5,7-pentaol (13) ($[\text{C}_6\text{H}_6]$ acetone)	83.8	73.3	38.0
heptamethyl ether (14)	82.4	73.3	37.1
octa-acetate (15)	79.4	71.5	36.1
(-) -Epicatechin derivatives			
(-) -Epicatechin (2) ($[\text{C}_6\text{H}_6]$ dimethyl sulphoxide)	77.9	64.7	28.1
(-) -Epicatechin (2) ($[\text{C}_6\text{H}_6]$ acetone)	79.4	66.9	29.1
3',4',5,7-Tetramethyl- (23) *	78.6	66.4	28.3
3-Acetyl-3',4',5,7-tetramethyl- *	77.2	67.9	25.8
3,3',4',5,7-Penta-acetyl-	76.6	66.6	26.0
(2 <i>R</i> ,3 <i>R</i> ,4 <i>R</i>)-3',4',5,7-Tetramethoxyflavan-3,4-diol (24)	74.9	70.7	63.5
(2 <i>R</i> ,3 <i>R</i> ,4 <i>R</i>)-4-(2,4,6-Trihydroxyphenyl)flavan-3,3',4',5,7-pentaol (8) ($[\text{C}_6\text{H}_6]$ acetone)	76.8	72.5	36.8
heptamethyl ether (9)	75.7	71.8	34.7
octa-acetate (10)	74.0	70.9	33.9
(2 <i>R</i> ,3 <i>R</i> ,4 <i>R</i>)-4-(2,4,6-Trihydroxy-3-methylphenyl)-flavan-3,3',4',5,7-pentaol ($[\text{C}_6\text{H}_6]$ acetone)	76.9	72.3	37.3
heptamethyl ether (11)	75.2 (d)	72.0}	35.5}
octa-acetate (12)	73.8	72.3}	34.6}
(2 <i>R</i> ,3 <i>R</i> ,4 <i>R</i>)-4-(2,4-Dihydroxyphenyl)flavan-3,3',4',5,7-pentaol hepta-acetate (7)	74.3	70.3	38.8
hexamethyl ether	72.5	70.4	36.4
(2 <i>R</i> ,3 <i>S</i> ,4 <i>S</i>)-4-Benzylthioflavan-3,3',4',5,7-pentaolpenta-acetate	74.2	70.2	38.0
	72.6	70.2	38.1

* Similar values recorded by K. Weinges, G. Schilling, W. Mayer, and O. Müller, *Annalen*, 1973, 1471.

and -7 as 4*R* has been made on the basis of a ^{13}C n.m.r. analysis of the flavan system (Tables 1 and 2). Assignments of signals to C-2, -3, and -4 to the flavan and tetra-

methoxyflavan systems were made on the basis of proton-uncoupled spectra and where necessary by selective proton decoupling.^{13,14} Several effects noted previously for cyclohexane and cyclohexene derivatives were also demonstrated for the flavan nucleus, but in general these were quantitatively diminished relative to the simpler ring systems. Thus Stothers, Roberts, Eliel, and their collaborators¹⁵⁻¹⁷ have shown that axial hydroxy-, methoxy-, and acetoxy-groups shield the attached carbon atom by *ca.* 5 p.p.m. relative to their equatorial counterparts in cyclohexane and thereby afford a direct means of stereochemical assignment. The relative shielding observed in flavan was much smaller, for a hydroxy-group [(1) and (2)]; (18) and (19)] 1.7—1.9 p.p.m., and for an acetoxy-group [acetates of (18)

TABLE 2

^{13}C N.m.r. chemical shifts of procyanidins (δ values; Me_4Si standard) *

	C-2	C-3	C-4	C-2'	C-3'	C-4'
Procyanidin B-1						
deca-acetate	73.5	70.9	34.0	78.3	68.4	27.1
Procyanidin B-2	76.9	72.9	36.9	79.3	66.4	29.6
deca-acetate	73.6	71.1	34.6	77.0	66.8	26.6
octamethyl ether	75.7	72.1 (d)	34.9 (d)	78.9	65.9	28.3
Procyanidin B-5	77.0	72.1	37.3	79.1	66.9	29.4
deca-acetate	73.7	70.8	34.7}	76.6	66.3	26.4
			34.9}			
Procyanidin B-3	83.5	73.2	38.0	82.0	68.3	28.2
deca-acetate	78.9	70.4	36.6	78.0	68.4	25.7
octamethyl ether	82.0	73.0	36.8	81.5	68.7	27.8
Procyanidin B-6						
deca-acetate	79.6	71.8	36.5}	77.6	68.4	26.1
			37.0}			
Procyanidin B-4	83.6	73.2	38.3	79.5	66.9	29.7
deca-acetate	78.9	70.0	36.6	77.0	66.6	26.5
Trimeric procyanidins						
Trimer C1	76.7	73.0	36.8	79.0	66.4	29.8
(32; $n = 1$)	76.7	72.1	36.8			

* Procyanidins in $[\text{C}_6\text{H}_6]$ acetone; derivatives in $[\text{C}_6\text{H}_6]$ chloroform.

and (19); acetates of (20 and (23)] 1.3—1.7 p.p.m. An additional feature of note in the flavan ring is the relatively small change in chemical shift of C-2 as compared with C-4 caused by introduction of a quasiequatorial (20) or quasiaxial (23) hydroxy- or acetoxy-group at C-3.

An observation of particular value for stereochemical assignments in six-membered ring systems is the upfield shift for the resonance of a carbon atom which is *gauche* to another carbon or hetero-atom at the γ -position.¹⁵⁻¹⁷ Similar results obtain in the flavan ring. Thus the C-2 resonance occurred at higher field when a quasiaxial hydroxy- or acetoxy-group was placed at C-4 than when this substituent was in the quasiequatorial position [Table 1, (18) and (19) and acetates, γ -effect 4.1—4.6 p.p.m.].

Introduction of an aryl group at C-4 in flavan resulted in deshielding of C-4 by 4.0—12.2 p.p.m.; generally the shift caused by a quasiequatorial group was greater than that of a quasiaxial aryl function. Similarly the

¹³ M. Tanabe, T. Hamesaki, D. Thomas, and Le Roy Johnson, *J. Amer. Chem. Soc.*, 1971, **93**, 273.

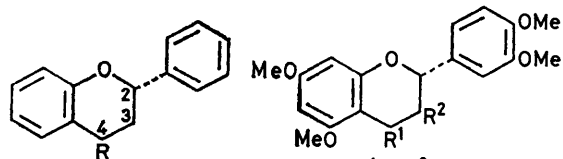
¹⁴ B. Birdsall, N. J. M. Birdsall, and J. Feeney, *J.C.S. Chem. Comm.*, 1972, 316.

¹⁵ J. D. Roberts, F. J. Weigert, J. Kroschawitz, and H. J. Reich, *J. Amer. Chem. Soc.*, 1970, **92**, 1338.

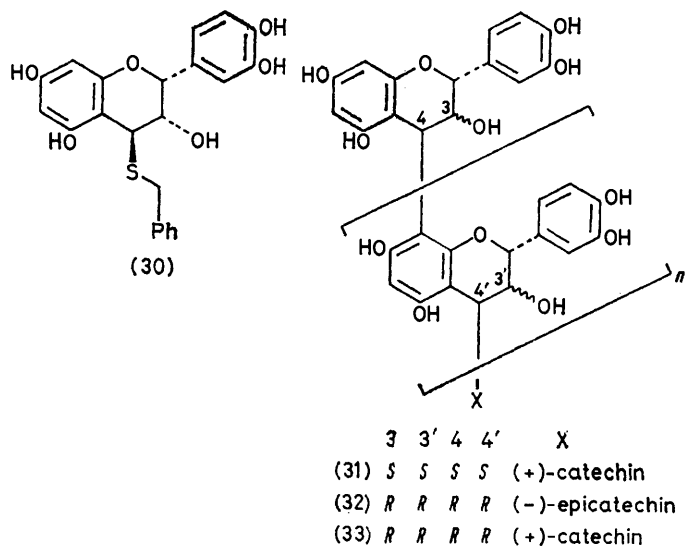
¹⁶ J. B. Stothers, 'Carbon-13 NMR Spectroscopy,' Academic Press, London and New York, 1972.

¹⁷ E. L. Eliel, W. F. Bailey, L. D. Kopp, R. L. Willer, D. M. Grant, R. Bertrand, K. A. Christensen, D. K. Dallin, M. W. Duch, E. Wenkert, F. M. Schell, and D. W. Cochran, *J. Amer. Chem. Soc.*, 1975, **97**, 322.

effects of a C-4 aryl group in the flavan system on the ^{13}C chemical shifts at the α - and β -positions were invariably smaller than those caused by phenyl or aryl substituents in cyclohexane.¹⁸ At the γ -position (C-2) effects entirely analogous to those observed with hydroxy- and acetoxy-groups were noted; these are of importance in the determination of absolute stereochemistry at C-4 in some of the procyanidin dimers. Where the C-4 aryl



(17)* R = H	(20) H OH (3 <i>S</i>)
(18)* R = β -OH	(21) OH OH (3 <i>S</i> , 4 <i>R</i>)
(19)* R = α -OH	(22) OH OH (3 <i>S</i> , 4 <i>S</i>)
	(23) H OH (3 <i>R</i>)
	(24) OH OH (3 <i>R</i> , 4 <i>R</i>)
	(25) H H



	3	3'	4	4'	X
(31)	<i>S</i>	<i>S</i>	<i>S</i>	<i>S</i>	(+)-catechin
(32)	<i>R</i>	<i>R</i>	<i>R</i>	<i>R</i>	(-)-epicatechin
(33)	<i>R</i>	<i>R</i>	<i>R</i>	<i>R</i>	(+)-catechin

group was placed in a quasiequatorial orientation a small downfield shift was observed [(13)—(15), 0.7—1.5 p.p.m.] but where the substituent was quasixial [(1), (6), and (8)—(12)] a γ -shielding effect (−2.5 to −5.9 p.p.m.) was noted. A direct comparison was possible with *cis*-(quasiequatorial) and *trans*-(quasixial) 4-phenylflavan and the shifts here were, respectively, +0.4 and −4.5 p.p.m.

The associated analysis of the ^{13}C n.m.r. data of the procyanidin dimers (Table 2) was based on that of the model systems outlined above and knowledge of the chemical composition of the dimer.^{5,6} Thus for procyanidin B-4 [(+)-catechin(−)-epicatechin] the signals due to C-2', -3', and -4' of the 'lower' flavan-3-ol unit were

assigned as in the monomer (−)-epicatechin (2) (Table 1). The remaining signals in the region 30—85 p.p.m. were then attributed to C-2, -3, and -4 of the 'upper' flavan-3-ol unit—in this case (+)-catechin. The analysis was completed by comparison with the appropriate model [(8) for procyanidins B-1, -2, and -5 and (13) for procyanidins B-3, -4, and -6]. In this way the absolute stereochemistry at C-4 in the dimers B-3, -4, and -6 was confirmed as *S*^{5,6} and that of the dimers B-1, -2, and -5 was established as *R* (a typographical error in ref. 4 notes this as *S*). Thus in these latter three dimers the ^{13}C signal for C-2 in the 'upper' flavan-3-ol showed a characteristic upfield γ -shift (Table 2, 2.5—3.1 p.p.m.) which has been related to the substitution of the 'upper' flavan-3-ol unit at C-4 by an aryl group (the 'lower' flavan-3-ol unit) in a quasixial orientation.

Earlier work has alluded to the possibilities of conformational isomerism in natural proanthocyanidins.^{8,19,20} In each case it was suggested that the observed ^1H n.m.r. phenomena were due to restricted rotation about the C(4)-aryl (sp^3 — sp^2) carbon-carbon linkage. During earlier work from this laboratory⁵ similar problems were encountered with the natural procyanidins although it was not clear whether the conformational isomerism was due to restricted rotation about the interflavan bond or to 'flipping' of the conformation of one of the heterocyclic rings in the natural product. A manifestation of these properties is undoubtedly encountered in the difficulties which attend crystallisation of the normal procyanidin derivatives (acetate or methyl ether).

A systematic investigation of these phenomena began with an examination of the model flavan-phloroglucinol adducts (5) and (6). ^1H N.m.r. data of the acetates were consistent with the adoption of a pseudochair form by the heterocycle with the phloroglucinol substituent at C-4 in a quasixial orientation.²¹ At ambient temperatures both acetates showed ^1H n.m.r. behaviour consistent with the presence of a restricted rotor in the molecule. Thus in [$^2\text{H}_5$] pyridine at −10 °C the triacetate (5) displayed three (methyl) acetate signals at δ 1.25, 1.64, and 1.86 but at +80 °C these had coalesced to two singlets at δ 1.55 (6 H) and 1.90 (3 H). Over the same temperature range the protons of the heterocycle gave rise to ^1H n.m.r. signals which remained unchanged—indicating that the shape of this ring remains fixed. These observations have therefore been interpreted in terms of restricted rotation around the flavan C(4)-phloroglucinol bond. Molecular models (CPK) suggest that this is caused primarily by steric interactions between the proton at C-2 and the π -system of ring A, and the substituents (acetoxy) *ortho* to the linkage on the phloroglucinol ring (26). Similar observations were made for the acetate in deuteriochloroform and by measurement of the usual parameters²² [T_c (temperature of coalescence) and $\Delta\nu$

²⁰ I. C. du Preez, A. C. Rowan, D. G. Roux, and J. Feeney, *Chem. Comm.*, 1971, 315.

²¹ J. W. Clark-Lewis, *Austral. J. Chem.*, 1968, **21**, 2059.

²² I. O. Sutherland, *Ann. Reports N.M.R. Spectroscopy*, 1971, **4**, 71.

¹⁸ T. P. Pehk and E. Lippmaa, *Org. Magnetic Resonance*, 1971, **3**, 679.

¹⁹ L. Jurd and R. Lundin, *Tetrahedron*, 1968, **24**, 2652.

(line separation)] the free energy of activation ΔG^*_{rot} for the conformational interconversion was determined as shown. The low temperature ^1H n.m.r. spectrum of the acetate (5) showed one phenolic acetate methyl signal at abnormally high field (δ 1.25) and this observation suggests that the preferred conformation adopted by the molecule and 'frozen out' at lower temperatures is that in which the plane of the phloroglucinol ring is aligned approximately with the flavan C(4)–C(4a) bond and perpendicular to the flavan nucleus. In this situation one of the *ortho*-acetate groups lies in the shielding region of the aromatic ring A of the flavan nucleus. Similar observations were made for the diacetate (6), and a corollary of this work therefore is the conformational stability of the heterocycle in 2,4-*trans*-4-arylflavans such that the 2-phenyl and 4-aryl group always remain respectively quasiequatorial and quasiaxial.

Derivatives (8)–(12) of the pentahydroxyflavan related in stereochemistry to (–)-epicatechin (2) showed ^1H n.m.r. behaviour entirely analogous to the flavan derivatives (5) and (6) and ΔG^*_{rot} data were adumbrated in the usual way. The origins of the hindrance to free rotation are assumed to be similar to that depicted (26) previously for (5) and (6). Significantly the peak intensities for the two rotamer populations of the trihydroxytoluene derivatives (11) and (12) although similar were not equal. That conformer in which the methyl group lies over the flavan ring A was least favoured and this may be ascribed to a 'buttressing' effect of the methyl group on the phenolic hydroxy-group *ortho* to the linkage. The resorcinol derivative (7)¹⁰ showed no evidence of conformational isomerism and hence two substituents *ortho* to the flavan C(4)–aryl link are a necessary structural feature for molecules to exhibit this property.

A complete kinetic treatment of the rotational process in the triacetate (5) by line-shape analysis²² gave ΔS^*_{rot} ($^{[2}\text{H}$]chloroform) -10.3 and ΔS^*_{rot} ($^{[2}\text{H}_5$]pyridine) -6.4 cal $\text{K}^{-1} \text{mol}^{-1}$ (the former value represents the result of refinement of the data reported earlier). This observation indicates that there is a substantial degree of organisation to reach the transition state for the rotational process and this factor therefore dictates that a strict comparison of the values of ΔG^*_{rot} for the derivatives (5)–(12) is not possible. However a broad generalisation of the data obtained indicates that for both the acetate and methyl ether derivatives conformational isomerism (as measured by ^1H n.m.r.) will be observed at ambient temperatures whilst for the less highly hindered phenols themselves this phenomenon will only become apparent at low temperatures. These results now provide a basis for the rationalisation of the ^1H n.m.r. properties of the natural procyanidin dimers with a 4*R*-configuration (B-1 and -7; B-2 and -5). The phenolic forms in $^{[2}\text{H}_6$]-acetone all give first-order ^1H n.m.r. spectra but below 0°C the spectra of two rotameric forms become apparent. In the acetate and methyl ether derivatives at ambient temperatures different rotameric forms may be distinguished. Thus in nitro- $^{[2}\text{H}_5$]benzene at 30 °C signals for the three aryl protons in the two rings A of procyanidin

B-2 deca-acetate are visible as two overlapping sets of signals owing to the presence of two rotameric forms (ratio *ca* 3:1). The major rotamer shows a double doublet (δ 6.15 and 6.24, J 2.0 Hz) and a singlet (δ 6.72), and the minor rotamer a different double doublet (δ 6.53 and 6.68, J 2.5 Hz) and singlet (δ 6.64). When the solution is heated to 160–180 °C the aryl proton signals collapse to give a first-order spectrum, a double doublet

TABLE 3

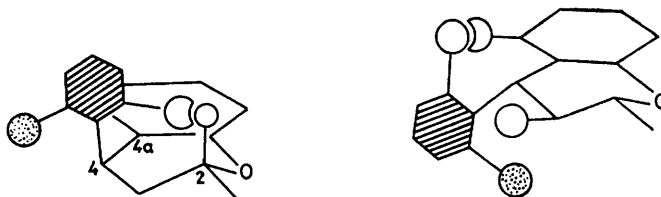
Estimated rotational barriers in natural procyanidins

Procyanidin	$\Delta G^*_{\text{rot}}/\text{kcal mol}^{-1}$
B-2	14.9 ^a
B-2 deca-acetate	19.5 ^b
B-2 octamethyl ether	18.7 ^b
B-3	19.1 ^c
B-3 deca-acetate	20.0 ^b
B-3 octamethyl ether	17.9 ^b
B-6 deca-acetate	19.8 ^b

^a In $^{[2}\text{H}_6$]acetone. ^b In nitro- $^{[2}\text{H}_5$]benzene. ^c In $^{[2}\text{H}_6$]-dimethyl sulphoxide.

(δ 6.35 and 6.39, J 2.0 Hz) and a singlet (δ 6.67). First-order analysis of all the acetate and methyl ether derivatives of procyanidins B-1 and -7 and B-2 and -5 is similarly possible at 160–180 °C in nitro- $^{[2}\text{H}_5$]benzene. Estimated values of ΔG^*_{rot} for the procyanidin B-2 derivatives are shown in Table 3.

The flavan (13) and its derivatives serve similarly as a model for the natural procyanidin dimers with a 4*S*-configuration. The free phenol (13), acetate (14), and methyl ether (15) all display temperature-dependent ^1H n.m.r. spectra; thus in each case the signals for the aromatic protons of the phloroglucinol ring (at C-4) appear as an AB quartet (J 2.5 Hz) at ambient temperatures, collapsing to a singlet as the temperature is raised. The signals associated with the aliphatic protons (at C-2,



(26)

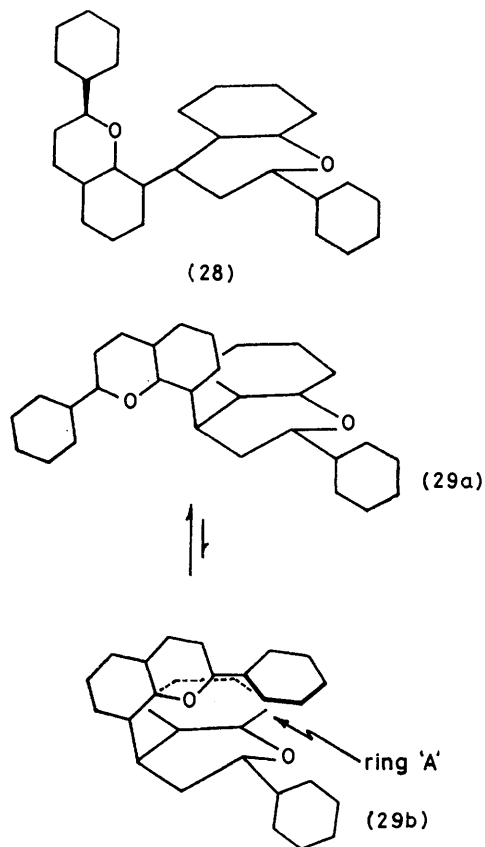
(27)

-3, and -4) were invariant over the same temperature range and the phenomenon has again been interpreted in terms of restricted rotation around the aryl-flavan C-4 linkage (27). This structural situation has several analogies, such as the 9-arylfluorene molecule,²³ and models (CPK) show that the oxygen substituents at C-3 and -5 and those in the *ortho*-positions on the phloroglucinol ring are primarily responsible for the steric interference to free rotation. Free energies of activation for the rotational process (ΔG^*_{rot}) were determined; the relatively high solvent-dependent barrier noted for the free phenol is worthy of comment. It seems probable that this is caused by hydrogen bonding of solvent molecules to the

²³ W. T. Ford, T. B. Thompson, K. A. J. Snoble, and J. M. Timko, *J. Amer. Chem. Soc.*, 1975, **97**, 95.

molecule around the C-4 aryl linkage. This relatively high energy barrier to free rotation is also found in the natural procyanidins (B-3 and B-6; B-4 and -8) with the 4*S*-configuration. At 30 °C ¹H n.m.r. spectra show the presence of two rotameric forms, but first-order spectra result at 100 °C in [²H₆]dimethyl sulphoxide. Similarly first-order spectra, consistent with the assigned structures, were obtained for the acetate and methyl ether derivatives at 160–180 °C in nitro[²H₅]benzene. Free energies of activation for the rotational barrier were estimated for procyanidin B-3 and procyanidin B-6 deca-acetate as shown in Table 3.

The general conclusion of this work is that although procyanidin dimers of the B group and their derivatives exhibit atropisomerism under the conditions of the ¹H



n.m.r. experiment, the energy barriers are too small to permit isolation of different conformational forms.⁸ These observations and an examination of molecular models nevertheless show that two energetically preferred conformations exist for each of the dimeric B group procyanidins. Those for the C(4)-C(6) linked (4*S*- or 4*R*-) dimers are of approximately equivalent energy and are very similar to those of the synthetic derivatives (8) and (13). On the other hand those for the C(4)-C(8) linked (4*S*- and 4*R*-) dimers are substantially different. Models show that, in their phenolic forms, (28) and (29a) are the preferred conformations for respectively the 4*S*- and 4*R*-configurations. However the alternative con-

formation (29b) is preferred for derivatives (acetate and methyl ether) of C(4)-C(8) linked dimers with the 4*R*-configuration. By using these assumptions a distinction between the C(4)-C(6) and C(4)-C(8) linked procyanidin dimers is now possible.

Biosynthetic studies show that the distinctive patterns of procyanidins found in plants arise directly by reaction of one or both of the flavan-3-ols (1) and (2) utilising their nucleophilic character at C-6 or C-8 with one or both of the carbocations (3) and (4). The reactions are under equilibrium control and can be reproduced exactly in the laboratory. Thus the pattern of di-, tri-, and tetra-meric procyanidins found in *Salix* sp. and *Fragaria* × *annanasa* is formed by acid-catalysed equilibration of (+)-catechin (1) and the carbocation (3), derived from the polymeric procyanidins (*a*–*c*) from *Salix caprea*, or procyanidin B-3. The predominant dimer formed is B-3 (*ca.* 85%) and a further dimer B-6 (*ca.* 10%) may be isolated and identified. Weinges, Perner, and Marx⁷ have synthesised procyanidin B-3 octamethyl ether diacetate and thus established that it has a C(4)-C(8) interflavan bond. This conclusion is supported by a comparison of the ¹H n.m.r. data for procyanidins B-3 and -6. Thus in the deca-acetate of procyanidin B-3 one conformational isomer (28) dominates the spectrum at 30 °C and a relative rotamer population of 4 : 1 was estimated. In contrast the corresponding derivative of the isomeric procyanidin B-6 shows the presence of two rotamers approximately equally populated. These observations are consistent (see above) with the assignment of a C(4)-C(6) link to procyanidin B-6 and a C(4)-C(8) link to procyanidin B-3. Analogously the procyanidins found in *Rubus idaeus* and *R. fruticosus*⁵ are formed by the reaction of (–)-epicatechin (2) with the cation (3). On the basis of identical evidence and arguments the major procyanidin dimer B-4 is the C(4)-C(8) linked and the minor one B-8 the C(4)-C(6) linked species.

The most commonly encountered pattern of procyanidins in nature is probably that characteristic of *Malus* sp., *Cotoneaster*, *Crataegus monogyna*,⁵ and this is reproduced in the laboratory by equilibration of the carbocation (4) [produced by the action of acid on the thioether (30), procyanidin B-2, or the polymeric procyanidins from *Crataegus monogyna* or the gum of *Butea frondosa*] with (–)-epicatechin (2). Arguments based on relative rotamer populations shown by the ¹H n.m.r. spectra lead to the conclusion that the major procyanidin dimer B-2 which is formed is C(4)-C(8) linked, and that dimer B-5 is C(4)-C(6) linked. Additional support for this assignment in the case of procyanidin B-2 derives from the distinctive high-field position of the AB quartet associated with the aryl ring A protons of the major rotamer (29b) of the ¹H n.m.r. spectrum of the deca-acetate.⁶ Acetylation of the phenolic groups in (–)-epicatechin (2), (+)-catechin, procyanidin B-5, and the models (8) and (11) produces a normal downfield shift of the two aryl ring A proton signals of 0.6–0.7 p.p.m.⁶ However in the major rotamer of the deca-acetate of procyanidin B-2 the ¹H n.m.r. spectrum shows an *upfield* shift of 0.15 p.p.m. for

one proton and a downfield shift of only 0.09 p.p.m. for the other. This however is readily rationalised in terms of the conformation (29b) for the deca-acetate in which the catechol ring of the 'lower' flavan unit lies directly over the aryl ring A of the 'upper unit' and produces a strong paramagnetic shielding effect on H-6 and -8. Finally, reaction of (4) with (+)-catechin (1) produces procyanidin B-1 [ca. 85%; C(4)-C(8) link] and procyanidin B-7 [ca. 10%; C(4)-C(6) link] and a pattern of procyanidins characteristic for example of the unripe seed coat of the cereal *Sorghum vulgare*. A noteworthy feature of the reactions of either cation (3) or (4) with phloroglucinol or flavan-3-ols (1) and (2) is that the products formed and isolated all have the C-4 aryl group *trans* to the hydroxy-group at C-3. No products (natural or synthetic) have been isolated in which the C-4 aryl group has a *cis*-relation to the hydroxy-group at C-3, but the factors which govern this preference are not clear.

Attention has recently become focused on the various polymeric forms of procyanidins found in plants (broadly groups *a* and *b* in the Robinsons' earlier classification²⁴). In some fruit they may constitute up to 4–5% *in toto* of the wet weight and preliminary observations indicate that the polymers fall into at least three categories based on solubility characteristics. Polymers of each of the three categories (*a*–*c*) have been isolated from the fruit of hawthorn (*Crataegus monogyna*) and from the male willow catkin (*Salix caprea*), and a polymer, assigned to class *a*, has been obtained from the seed coat of *Sorghum vulgare*. In some cases it was not found possible to free polymers of classes *b* and *c* from material of a protein nature. Polymers of class *a* are completely degraded by toluene- α -thiol-acetic acid,⁵ and it seems reasonable to assume that they are formed *in vivo* from the continued polymerisation of the appropriate dimer(s) with further cation (3) or (4) intermediate [*e.g.* hawthorn: procyanidin B-2 and (4) to give (32); willow: procyanidin B-3 and (3) to give (31); *Sorghum*: procyanidin B-1 and (4) to give (33)]. Measurement of the ratio of (–)epicatechin (2) to (2*R*, 3*S*, 4*S*)-4-benzylthioflavan-3,3', 4', 5, 7,-pentaol (30) derived from the hawthorn polymer gave a value of 1 : 3.5, which suggests structure (32; *n* = 3 or 4) and a number-average molecular weight of about 1 300. In contrast, degradation of the *Sorghum* polymer gave a ratio of (+)-catechin to (30) of 1 : 6.5, which leads to structure (33; *n* = 6 or 7) and an average molecular weight of approximately 2 175. In the case of polymers of classes *b* and *c* from willow catkins both of these are only partially degraded by toluene- α -thiol and by phloroglucinol and mineral acid. This difference suggests different structural patterns in these polymers but further work is desirable to substantiate this view.

Models (CPK) of procyanidin polymers of class *a*, constructed in the light of the conformational restraints

²⁴ G. M. Robinson and R. Robinson, *J. Chem. Soc.*, 1935, 744.

²⁵ J. W. Clark-Lewis and R. W. Jemison, *Austral. J. Chem.*, 1970, **23**, 320.

²⁶ E. T. Østensen, *Acta Chem. Scand.*, 1975, **29B**, 1067.

²⁷ M. J. Betts, B. R. Brown, and M. R. Shaw, *J. Chem. Soc.*, 1969, 1178.

discussed above for the dimeric procyanidins, show a thread-like structure in which the central linear core is composed of rings A and B of the flavan-3-ol repeat unit. The 3', 4'-dihydroxyphenyl units (ring C) project laterally from this core and their arrangement describes a regular helical conformation; *that of polymers such as (31) derived from (3) is a helix with a right-hand screw and that of polymers such as (32) and (33) derived from (4) is a helix with a left-hand screw*. Whilst there is yet no evidence to suggest that these helical structures have any biological or evolutionary significance they are a further interesting example of the economy of nature. Thus different plants have evolved the means of synthesis of isomeric polymers with opposite helicities by the simple expedient of a change of stereochemistry of the hydroxy-group at C-3 in the carbocation precursor, (3) or (4).

EXPERIMENTAL

Mass spectra were obtained with A.E.I. MS9 and MS12 instruments. ¹H N.m.r. spectra were recorded at 100 MHz with a Varian HA-100 spectrometer and ¹³C n.m.r. spectra at 25.15 MHz with a JEOL PFT-100 spectrometer. Paper chromatographic analysis and the isolation of natural procyanidins were carried out as previously described.⁵ The following compounds were prepared according to previously described procedures: 2,4-*cis*-flavan-4-ol,²⁵ 2,4-*trans*-flavan-4-ol,²⁵ flavan,²¹ 4-(2-methoxyphenyl)flavan-4-ol,¹⁹ 2,4-*cis*-4-phenylflavan,²⁶ 2,4-*trans*-4-phenylflavan,²⁶ (2*R*,3*S*,4*S*)- and (2*R*,3*S*,4*R*)-3',4',5,7-tetramethoxyflavan-3,4-diol,¹⁹ (2*R*,3*R*,4*R*)-3',4',5,7-tetramethoxyflavan-3,4-diol,²⁷ mollisacacidin,²⁸ 2,4-*cis*-acetoxylflavan,²⁵ and 3',4',5,7-tetramethoxyflavan.²⁹

Isolation of Procyanidin Polymers.—(i) *Willow catkin* (*Salix caprea*). Male willow catkins (March; 1 000 g) were extracted as previously described⁵ to give the ethyl-acetate-soluble phenols, a residual aqueous extract, and insoluble plant debris.

(a) Chromatography of the ethyl acetate-soluble phenols (20.0 g) on Sephadex LH-20 and elution with ethanol (3 500 ml) gave the di- and tri-meric procyanidins. Elution then with methanol (1 500 ml) gave after evaporation polymer (*a*) as a pale brown powder (1.8 g) (Found, after drying at 80 °C and 0.01 mmHg for 48 h: C, 58.3; H, 4.8%). The polymer was degraded with toluene- α -thiol and acetic acid⁵ to give principally (+)-catechin and a mixture of thioethers [(2*R*,3*R*,4*R*)-4-benzylthioflavan-3,3',4',5,7-pentaol], which were separated by chromatography on Sephadex LH-20.⁵ Measurement of the ratio of (+)-catechin to the thioethers indicated a formula such as (32; *n* = 3–4) for the polymer.

(b) The insoluble plant debris remaining after methanolic extraction was re-extracted by stirring with acetone-water (1 : 1; 2 × 1 000 ml) for 48 h at room temperature. Removal of the plant tissue by filtration and washing with acetone-water (1 : 1; 1 000 ml) afforded a residue which no longer gave a positive procyanidin test (ethanol, H⁺; 60 °C). The filtrate was concentrated (to ca. 500 ml) and set aside for 48 h. The precipitate was filtered off and reprecipitated from the same solvent mixture to give polymer *c* (0.5 g) as a pale buff powder (Found, after drying at 80 °C and 0.05 mmHg for 48 h: C, 55.9; H, 5.3; N, 1.2; S, 0.4%).

A further polymer giving a positive procyanidin test was

²⁸ S. E. Drewes and D. G. Roux, *Biochem. J.*, 1965, **94**, 482.

²⁹ J. W. Clark-Lewis and R. W. Jemison, *Austral. J. Chem.*, 1968, **21**, 2247.

isolated from the aqueous residue following the initial methanolic extraction.⁵ It was very hygroscopic and contained ca. 30% protein material.

(ii) *Hawthorn* (*Crataegus monogyna*). Ripe hawthorn berries (September; 1 000 g) were extracted with methanol and treated as above to give the ethyl acetate-soluble phenols (ca. 20 g), an aqueous residue, and insoluble plant debris. Two analogous polymers were isolated as described for willow; polymer *a* (after chromatography of the ethyl acetate-soluble phenols) (2.8 g) (Found, after drying at 80 °C and 0.05 mmHg for 48 h: C, 56.2; H, 4.4%); polymer *c* (from acetone-water extraction of the insoluble plant debris) (4.8 g) (Found: C, 59.7; H, 5.3%). Polymer *a* was degraded by toluene- α -thiol and acetic acid⁵ to give (–)-epicatechin and (2*R*,3*S*,4*S*)-4-benzylthioflavan-3,3',4',5,7-pentaol, which were separated by chromatography on Sephadex LH-20.⁵ Measurement of the ratio of (–)-epicatechin to the thioether indicated a formula such as (32; $n = 3$ or 4) for the polymer.

(2*R*,3*S*,4*S*)-4-(2,4,6-Trihydroxyphenyl)flavan-3,3',4',5,7-pentaol.—Willow procyanidin polymer (type *c*; 4.0 g) and phloroglucinol (5.0 g) in dioxan-water (1 : 1 v/v containing hydrochloric acid to give 0.5M) were stirred at 20 °C for 24 h. The mixture was then diluted with water (250 ml) and extracted with ethyl acetate (6 × 150 ml). Removal of the organic solvent left a gum which was chromatographed on Sephadex LH-20 (5 × 40 cm) in ethanol. Fractions (15 ml) 27–50 were combined and evaporated to give a gum. Rechromatography in the same system and finally with acetone as eluant gave the *product* as a light buff powder (0.48 g) (Found: C, 60.5; H, 4.7. C₂₁H₁₈O₉ requires C, 60.9; H 4.3%), R_F (A) 0.57, R_F (B) 0.54. The ¹H n.m.r. spectrum ([²H₅]acetone) showed at –30 °C the two aromatic protons of the C-4 phenyl substituent as an AB system (δ 5.87 and 5.99); at 30 °C they had collapsed to a broad singlet (δ 5.95) and at 55 °C to a sharp singlet (δ 5.98); T_c 30 °C; $\Delta\nu$ 12 Hz; in (CD₃)₂SO T_c 44 °C, $\Delta\nu$ 11 Hz.

Methylation of the phenol (diazomethane in ether-ethanol) gave, after preparative t.l.c. [ethyl acetate-light petroleum (b.p. 60–80 °C), 1 : 1; R_F 0.35] and crystallisation from methanol, the heptamethyl ether as needles, m.p. 196–197°, mixed m.p. 197–198° (with the *product* prepared by the procedure of Jurd and Lundin¹⁹) (Found: C, 65.6; H, 6.5. Calc. for C₂₈H₃₂O₉: C, 65.6; H, 6.3%); [α]₅₇₈²⁰ –194° (c 0.6 in CHCl₃); M^+ 512 and fragment ions at *m/e* 344, 333, and 301. ¹H N.m.r. spectra were determined in [²H₅]pyridine-[²H]chloroform (1 : 1) at various temperatures; values for $\Delta G^{\ddagger}_{rot}$ were determined by observation of signals of the 4-(2,4,6-trimethoxyphenyl) substituent; for the AB system of the two aromatic protons (δ 6.07 and 6.22, J 2.0 Hz), T_c 40 °C; for the 2- and 6-methoxy-signals (δ 3.49 and 3.94), T_c 50 °C.

The *octa-acetate* (acetic anhydride-pyridine) crystallised from methanol-water and finally methanol as small white prisms, m.p. 113–115° (Found: C, 59.0; H, 4.6. C₃₇H₃₄O₁₇ requires C, 59.2; H, 4.6); [α]₅₇₈²⁰ –70.0 (c 0.7 in CHCl₃); M^+ 750. ¹H N.m.r. spectra at various temperatures were determined in nitro[²H₅]benzene and values of $\Delta G^{\ddagger}_{rot}$ were determined from observations on the phloroglucinol 2- and 6-acetate methyl signals; $\Delta\nu$ 43 Hz, T_c 125 °C.

(2*R*,3*R*,4*R*)-4-(2,4,6-Trihydroxyphenyl)flavan-3,3',4',5,7-pentaol.—*Butea frondosa* gum (100 g) was stirred in water (400 ml) at 60 °C for 4 h. The solution was filtered and allowed to cool to room temperature. Phloroglucinol (15 g) was added, followed by concentrated hydrochloric acid (20 ml). The deep red solution was stirred overnight at room

temperature, sodium chloride (20 g) was added, and the solution was then extracted with ethyl acetate (6 × 250 ml). The extract was passed through a pad of animal charcoal (Norit) and evaporated to leave a gum, which was chromatographed in ethanol on Sephadex LH-20 (3.5 × 40 cm). Fractions (15 ml) 30–80 were combined to give a solid. Rechromatography on the same adsorbent in acetone finally gave the *product* as an off-white solid (1.4 g) (Found: C, 57.8; H, 4.6. C₂₁H₁₈O₉·H₂O requires C, 58.3; H, 4.6%); R_F (A) 0.56, R_F (B) 0.52; [α]₅₇₈²⁰ +106.3° (c 0.56 in MeOH).

The *octa-acetate* (acetic anhydride-pyridine) crystallised from ethanol as small white plates, m.p. 148–150° (Found: C, 59.0; H, 4.5. C₃₇H₃₄O₁₇ requires C, 59.2; H, 5.4%); M^+ 750; [α]₅₇₈²⁰ +93.6° (c 0.22 in CHCl₃). The ¹H n.m.r. spectrum (nitro[²H₅]benzene) at 30 °C showed the phloroglucinol H-3 and -5 signals as a double doublet, δ 6.84 and 6.96, J 1.9 Hz, which had collapsed at 120 °C to a singlet, δ 6.89; T_c 95 °C; $\Delta\nu$ 12 Hz (these values were used to determine $\Delta G^{\ddagger}_{rot}$).

The *heptamethyl ether* was prepared by treatment with ethereal diazomethane and preparative t.l.c. (ethyl acetate-hexane, 7 : 3) as a white granular solid (from ethyl acetate-hexane), m.p. 102–104° (Found: C, 65.6; H, 6.3. C₂₈H₃₂O₉ requires C, 65.6; H, 6.3%); M^+ 512. The ¹H n.m.r. spectrum ([²H]chloroform-[²H₅]pyridine, 1 : 1) at –30 °C showed the phloroglucinol H-3 and -5 signals as a double doublet, δ 6.09 and 6.23, J 1.3 Hz, which had collapsed at 100 °C to a singlet, δ 6.12; T_c 55 °C; $\Delta\nu$ 14 Hz. A minor *product* obtained from fractions 90–180 was the *phenol* (C 61.0; H, 4.8. C₂₆H₃₀O₁₅ requires C, 61.5; H, 4.3%); [α]₅₇₈²⁰ +129.5° (c 0.4 in MeOH), R_F (A) 0.48, R_F (B) 0.39.

Reaction of Flavan-4-ol with Phloroglucinol.—2,4-*cis*-Flavan-4-ol (3 g) and phloroglucinol (6 g) were dissolved in absolute ethanol (100 ml) and a stream of anhydrous hydrogen chloride was passed through for 15 min. After 12 days at room temperature the mixture was poured into water (1 000 ml) and extracted with chloroform (4 × 300 ml). Removal of the solvent after drying (MgSO₄) gave a gum which was treated with acetic anhydride (20 ml) and pyridine (20 ml) for 24 h. The mixture was added to ice-water and after 2 h the solid was collected. Preparative t.l.c. (silica; benzene-acetone, 10 : 1) gave three components, (i) R_F 0.55, (ii) 0.62, and (iii) 0.65.

(i) 2,4-*trans*-4-(2,4,6-Triacetoxyphenyl)flavan (0.95 g) re-crystallised from methanol as needles, m.p. 190–191° (Found: C, 70.3; H, 5.6. C₂₇H₂₄O₇ requires C, 70.5; H, 5.2%); M^+ 460. The ¹H n.m.r. spectrum ([²H]chloroform) at 30 °C showed the acetate methyl signals as a sharp singlet δ 2.19 and two broad singlets, δ ca. 1.6 and 1.85. At –8 °C the latter two signals appeared as sharp singlets, δ 1.89 and 1.52, and at 52 °C as a broad singlet, δ 1.71; T_c 37 °C; $\Delta\nu$ 37 Hz. A complete line-shape analysis in [²H]chloroform²² gave the following values of $\ln k/T$: –1.1 °C, –4.278; 9.2 °C, –3.300; 25 °C, –2.102; 33.8 °C, –1.500; 41 °C, –1.080; 51.9 °C, –0.429. In [²H₅]pyridine values of $\ln k/T$ were: 15.2 °C, –2.15; 24.2 °C, –1.417; 35.4 °C, –0.6; 44 °C, –0.006; 55.5 °C, 0.65.

(ii) 2,4-*trans*-4-(2,6-Diacetoxy-4-ethoxyphenyl)flavan (0.38 g) crystallised as needles from methanol, m.p. 151–152° (Found: C, 68.6; H, 5.2. C₂₇H₂₆O₆ requires C, 68.5; H, 5.5%); M^+ 474. The ¹H n.m.r. spectrum ([²H]chloroform) showed the acetoxyethyl signals as two singlets (δ 1.60 and 1.89) at –24 °C and as a singlet (δ 1.74) at 60 °C; T_c 37 °C; $\Delta\nu$ 29 Hz.

(iii) 1,3,5-Triacetoxy-2,6-diflavan-4-ylbenzene was isolated as small needles (0.1 g) (from ethanol), m.p. 195–196° (Found: C, 75.3; 4, 5.7. $C_{42}H_{36}O_8$ requires C, 75.7; H 5.4%); M^{++} 668.

Biogenetically Patterned Synthesis.—(i) *Procyanidin B-2*. A solution of (–)-epicatechin (1.1 g, 3.8 mol) and (2*R*,3*S*,4*S*)-4-benzylthioflavan-3,3',4',5,7-pentaol (0.7 g, 1.7 mmol) in dioxan (20 ml) containing water (16 ml) and concentrated hydrochloric acid (4 ml) was stirred at room temperature for 48 h. It was then concentrated at 30 °C, diluted with water (50 ml), and extracted with ethyl acetate (6 × 60 ml). Evaporation gave a gum which was chromatographed in ethanol on Sephadex LH-20 (30 × 4 cm column); fractions (12 ml) were collected. Fractions 80–105 gave procyanidin B-2 (0.45 g), identified by paper chromatography, 1H n.m.r., and conversion into the deca-acetate.⁵ Fractions 130–175 were combined to yield, after evaporation, a solid (150 mg). This was subjected to counter-current distribution between ethyl acetate and water (25 ml phase volume; 50 transfers). Tubes 32–38 gave, on work-up, procyanidin B-5 (0.05 g), identified by paper chromatography, 1H n.m.r., and conversion into its deca-acetate. Tubes 3–10 gave the trimer C-1⁵ (0.04 g).

Similar experiments were conducted with procyanidin B-2 (1.5 g) and (–)-epicatechin (1.0 g) or *Butea frondosa* gum (5.0 g) and (–)-epicatechin (1.0 g). Identical results were obtained. When *Butea frondosa* gum was used the dioxan (solvent) was omitted, and the reactants were dissolved by heating in water (60 ml) prior to addition of concentrated hydrochloric acid (6 ml).

(ii) *Procyanidin B-1*. A solution of (+)-catechin (1.2 g) and (2*R*,3*S*,4*S*)-4-benzylthioflavan-3,3',4',5,7-pentaol (0.8 g) in dioxan (20 ml) containing water (16 ml) and concen-

trated hydrochloric acid (4 ml) was treated as in (i). Fractions 85–102 yielded procyanidin B-1 (0.38 g), identified by paper chromatography, 1H n.m.r., and conversion into the deca-acetate, m.p. and mixed m.p. 228–229°. A similar experiment with *Butea frondosa* gum (5.0 g) and (+)-catechin (0.9 g) gave an identical result.

(iii) *Procyanidin B-3*. Polymer (*Salix caprea*, c; 5.0 g) and (+)-catechin (1.2 g) were dissolved in 0.5*M*-hydrochloric acid (30 ml in dioxan–water, 1 : 1). The solution was stirred for 48 h at room temperature, concentrated at 30 °C, diluted with water (100 ml), and extracted with ethyl acetate (6 × 75 ml). Evaporation gave a gum which was chromatographed on Sephadex LH-20 (30 × 4 cm column); fractions (10 ml) were collected. Fractions 88–125 gave procyanidin B-3 (0.43 g), identified by paper chromatography, 1H n.m.r., and conversion into the deca-acetate. Fractions 140–200 gave impure procyanidin B-6 (0.12 g). Acetylation (acetic anhydride–pyridine) and preparative t.l.c. (silica; benzene–acetone, 4 : 1) gave the major product (R_F 0.48). Crystallisation from methanol–water gave *procyanidin B-6 deca-acetate* (0.06 g) as small prisms, m.p. 142–144° (Found: C, 59.9; H, 4.8. $C_{50}H_{46}O_{22}$ requires C, 60.1; H, 4.6), $[\alpha]_{589}^{20}$ –19.9° (*c* 0.7 in $CHCl_3$).

A similar experiment, with identical results, was carried out with a procyanidin B-3 (1.3 g) and (+)-catechin (0.8 g)

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