

Synthesis and Conformation of Procyanidin Diastereoisomers

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The four theoretically possible diastereoisomeric 4 \rightarrow 8 linked procyanidin dimers with two 2,3-*cis*-flavanoid units, epicatechin-(4 β \rightarrow 8)-epicatechin (6), epicatechin-(4 β \rightarrow 8)-*ent*-epicatechin (8), *ent*-epicatechin-(4 α \rightarrow 8)-epicatechin (7), and *ent*-epicatechin-(4 α \rightarrow 8)-*ent*-epicatechin (2), have been synthesized together with the 2,3-*trans* diastereoisomers catechin-(4 α \rightarrow 8)-catechin (14) and catechin-(4 α \rightarrow 8)-*ent*-catechin (15). The preferred rotamer conformations of the deca-acetate derivatives of (6), (8), (14), and (15) in chloroform were deduced from their ^{13}C n.m.r. and high-field ^1H n.m.r. spectra. The ^1H n.m.r. spectra of the preferred conformers of the pairs of acetate derivatives of (6) and (15), and (8) and (14), were qualitatively similar as the relative configuration about the interflavanoid bond of one pair has a *meso*-relationship to the other.

Procyanidin dimers provide an interesting example of natural products which possess detectable conformational isomers.^{1,2} Steric interactions between substituents in the vicinity of the interflavanoid bond slow rotational exchange sufficiently for the ^1H or ^{13}C n.m.r. spectra of the dimers, together with their deca-acetate and octamethyl ether derivatives, to exhibit separate signals for each conformational isomer.¹ The factors controlling the relative populations and energy barriers between the two conformational isomers have already been discussed.^{1,2} However, it may be briefly noted that two situations exist for procyanidin dimers with a single interflavanoid bond. Those with a procyanidin (PC) unit with a 2,3-*cis*-configuration have the appending flavan-3-ol unit disposed with a pseudo-axial orientation, and consequently the barrier to rotation about the interflavanoid bond is considerably lower than in those dimers with a 2,3-*trans*-configuration, where the appending pseudo-equatorial flavan-3-ol unit suffers considerable steric interaction with the 5- and 7-substituents.^{1,2} This study pursues this phenomenon further and provides some more examples of conformational isomerism in procyanidin dimers.

Synthesis of Procyanidin Diastereoisomers.—Virtually all proanthocyanidins so far isolated possess units with a (2*R*) configuration.³ However, as noted in a preliminary communication,⁴ there are exceptions. In particular, procyanidin polymers isolated from the palms *Phoenix canariensis* and *Rhopalostylis sapida* contain 2,3-*cis* PC units with both a (2*R*) and (2*S*) configuration.⁴ Earlier, *ent*-epicatechin (1) and *ent*-epicatechin-(4 α \rightarrow 8)-*ent*-epicatechin (2)† had been isolated from *Chamaerops humilis*.^{6,7} As reported in the earlier communication,⁴ calculation from the specific rotation of the intact polymer, or chemical degradation, showed that the polymer consisted of both (2*R*)- and (2*S*)-PC units in the ratio 1 : 3. Experimental details of this work are given in this paper.

Further work⁸ has established that such mixed polymers are relatively widely distributed in species in the Monocotyledonae, so that the capacity to produce units with mixed stereochemistry is not confined to the Palmae.

The isolation of a PC polymer with both (2*R*)- and (2*S*)-2,3-*cis* units provided a unique opportunity to synthesize a series of diastereoisomers of a PC dimer. As had been established earlier,^{1,9} PC polymers may be degraded in mild acid solution to monomeric 5,7,3',4'-tetrahydroxyflavan-3-ol 4-carbocations [(3) and (4) in Scheme 1], which may be captured stereo- and regio-specifically^{1,10,11} by a flavan-3-ol unit to produce pre-

dominantly a 4 \rightarrow 8 linked dimer (see Scheme). In particular, reaction of the palm polymer with epicatechin (5) produced a mixture of the diastereoisomeric dimers epicatechin-(4 β \rightarrow 8)-epicatechin (6) and *ent*-epicatechin-(4 \rightarrow 8)-epicatechin (7). Similarly *ent*-epicatechin (1) produced the diastereoisomers epicatechin-(4 β \rightarrow 8)-*ent*-epicatechin (8) and *ent*-epicatechin-(4 α \rightarrow 8)-*ent*-epicatechin (2). Each reaction mixture was separated initially on Sephadex LH-20¹² and the 4 \rightarrow 8 dimer fraction separated into its constituent diastereoisomers by reverse-phase h.p.l.c.⁵

The pairs of diastereoisomers (2) and (6), and (7) and (8), are enantiomers. This was confirmed by the close similarity of each pair's chiroptical and n.m.r. properties for both the phenols and their deca-acetate derivatives. The dimeric nature of the diastereoisomers was confirmed by mass spectroscopy of the deca-acetate derivatives, which all gave a spectrum containing a characteristic m/z 938 ($M^+ - \text{HOAc}$) ion.

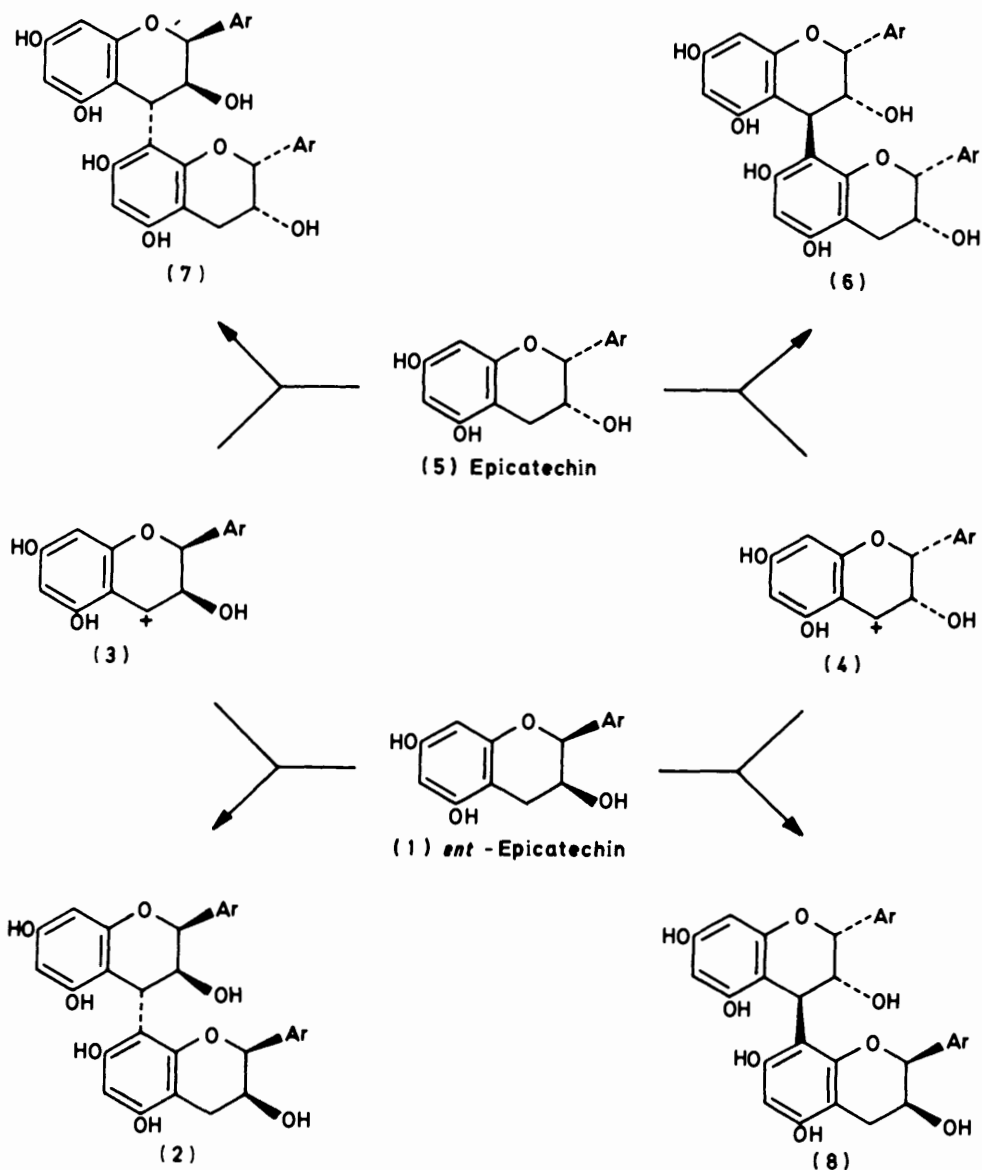
Larger amounts of (8) were synthesized by reaction between horsechestnut polymer [containing only (2*R*)-2,3-*cis*-PC units¹³] and *ent*-epicatechin (1). This also enabled isolation of epicatechin-(4 β \rightarrow 6)-*ent*-epicatechin (9) which is diastereoisomeric with procyanidin B5 [epicatechin-(4 β \rightarrow 6)-epicatechin (10)].

A further pair of 4 \rightarrow 8 linked diastereoisomers containing (2*R*)-2,3-*trans*-PC units were synthesized by reaction in mild acid between the 2,3-*trans*-5,7,3',4'-tetrahydroxyflavan-3-ol 4-carbocation (11) and catechin (12) or *ent*-catechin (13) to form the dimers catechin-(4 α \rightarrow 8)-catechin (14) and catechin-(4 α \rightarrow 8)-*ent*-catechin (15), respectively. The 2,3-*trans*-carbocation (11) was formed by reduction of (2*R*,3*R*)-(+)-taxifolin (16) with sodium borohydride.¹⁴ Decomposition in mild acetic acid produces (2*R*,3*S*,4*R*)-(+)-3,4,5,7,3',4'-hexahydroxyflavan (17)¹⁵ which readily forms the 4-carbocation (11) in 0.1*M*-hydrochloric acid.^{14,15} The dimers (14) and (15) were isolated and purified as described for the 2,3-*cis* dimers.

Synthesis of the above diastereoisomers required preparation of several grams each of *ent*-catechin (13) and *ent*-epicatechin (1). Current literature,^{16,17} while indicating that these compounds may be obtained by epimerisation of epicatechin (5) and catechin (12) respectively, does not give a full account of the factors which control the optimum yield of the reaction, which are as follows.

Epimerisation of Catechin and Epicatechin.—The facile epimerisation of catechin (12) to a mixture of itself and *ent*-epicatechin (1), or epicatechin (5) to a mixture of itself and *ent*-catechin (13) in basic or neutral solution is well established.^{16,17} The mechanism proposed by Whalley¹⁸ proceeding

† The proanthocyanidin nomenclature used in this paper is described in detail elsewhere.⁵



Scheme. Synthesis of 2,3-*cis*-procyanidin diastereoisomers. Ar = C₆H₃(OH)_{2-3,4}

through ionization of the 4'-hydroxy group and a quinone methanide intermediate, is supported by the fact that catechin tetramethyl ether remains unchanged after prolonged heating in alkaline solution (the earlier observation of Whalley¹⁸ has been independently corroborated in this study), and also that no epimerisation takes place in acid solution (such as the conditions used to synthesize the dimers).

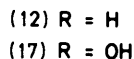
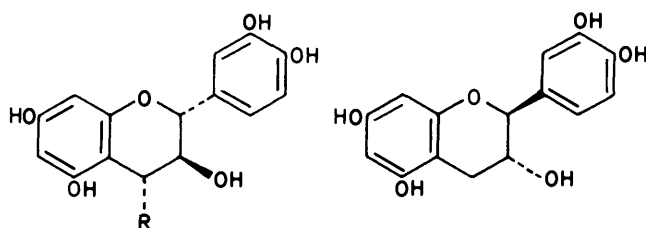
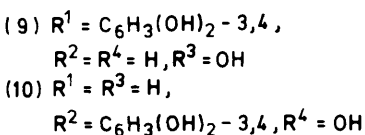
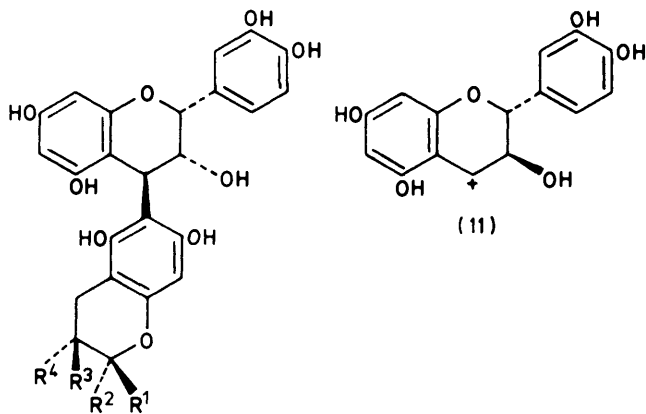
More recent studies have shown that, at pH 11.0 at 57 °C, for example, catechin (12) is converted into *ent*-epicatechin (1) with a half-life of 2.6 min whereas the reverse reaction from epicatechin (5) to *ent*-epicatechin (1) is about three times faster.¹⁹ This implies that the ratio of catechin to *ent*-epicatechin is about 3 : 1 at equilibrium, which has been corroborated, both by ourselves²⁰ and by Kiatgrajai and Welton,¹⁹ by measurements of the relative concentrations of these products at equilibrium by h.p.l.c. which gave a value of 74 : 26. Catechic acid²¹ is formed in a parallel reaction from catechin or epicatechin at *ca.* one fifth of the rate of the epimerisation reaction.¹⁹

It was therefore concluded that the *ent*-isomers of catechin

or epicatechin could be formed efficiently by either *brief* treatment with a strongly basic solution, and rapid quenching of the reaction, or prolonged heating in neutral solution. Both synthetic methods were used in this study.

Conformational Studies of the Diastereoisomers.—Dreiding models showed that the conformation of dimers composed of flavanoid units with the same absolute configuration at C-2, *i.e.* (2*R*),(2'*R*) or (2*S*),(2'*S*), referred to subsequently as 'normal' dimers, were different from those composed of units with the opposite absolute configuration at C-2, *i.e.* (2*R*),(2'*S*) or (2*S*),(2'*R*), referred to subsequently as 'crossed' dimers (the numbering for the procyanidin dimers used in this paper is given in Figure 1). These stereochemical differences were reflected in the n.m.r. spectra of the dimers, especially the ¹H n.m.r. of the deca-acetate derivatives, and enabled establishment of the preferred conformations of the procyanidins.

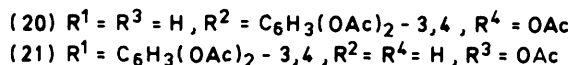
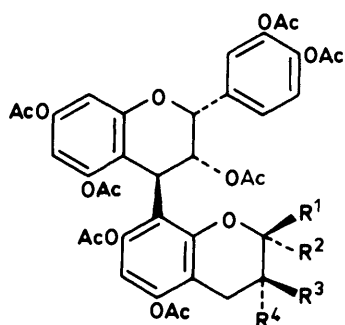
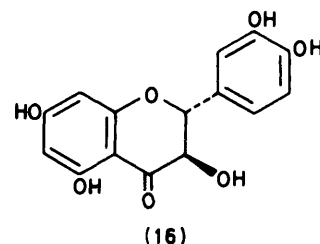
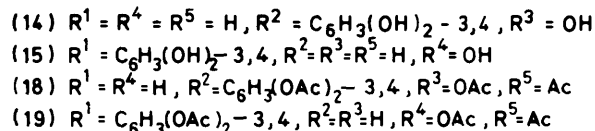
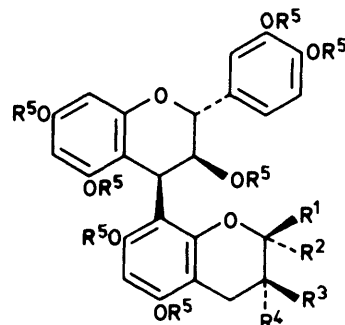
The procyanidin dimers are examples of sp³-sp² C-C single bond rotors.²² Whereas the low energy states of sp³-sp³ rotors are invariably assumed to be staggered (or near staggered)



conformations, while the high energy form is eclipsed, the energy profile about an sp^3-sp^2 bond depends on the nature of the substituents.²² When observed, the procyanidin dimer rotational isomers display two sets of n.m.r. signals,¹ and therefore are examples of single-bond rotors with a double lower-energy state.²² These lower-energy states have the conformations shown in Figure 2 for 4 \rightarrow 8 linked 2,3-*cis*- and 2,3-*trans*-procyanidin unit dimers, where the upper (U) flavanoid unit has an absolute configuration of (2*R*) at C-2. [If the U-unit was (2*S*) the conformations for the 2,3-*cis* and -*trans* dimers would be reversed.] The preferred low-energy (a) and (b) conformations (in common with many other similar examples²²) correspond to the C-4 proton eclipsing the aromatic A-ring of the lower flavanoid unit, and to the bulky C-2 and C-4a substituents lying in the position of least steric interaction with the lower unit. The pair of isomers 2,3-*cis* (b) and 2,3-*trans* (b) correspond to a conformation with minimum overlap of the flavanoid units, whereas 2,3-*cis* (a) and 2,3-*trans* (a) are positioned so that there is some overlap of the heterocyclic- and B-ring of the U-unit with the L-unit A-ring. As pointed out by Haslam and co-workers, there is significant steric interaction in the 2,3-*cis* (a) conformer between the U-unit and 2-H and A-ring π -electrons of the L-unit which result in the shielding effects observed in the deacacetate derivative of epicatechin-(4 β \rightarrow 8)-epicatechin (6).

Interestingly the pairs of rotational isomers 2,3-*cis* (a) and 2,3-*trans* (b), and 2,3-*cis* (b) and 2,3-*trans* (a) (Figure 2) have a pseudo-enantiomeric relationship, a feature alluded to earlier by Haslam.²

(a) *Phenol spectra.* As observed earlier, it has been established experimentally that the energy barrier to rotation in 2,3-*cis*-procyanidin dimers is considerably less than in 2,3-



trans dimers, the free energy of rotation in the latter compounds being similar to those observed for 9-fluorene derivatives because of the buttressing effect of the 5- and 3-oxygen substituents on the U-unit,¹ producing severe steric interactions with the L-unit in the high-energy rotational transition state.²² Consequently conformationally averaged n.m.r. spectra for epicatechin-(4 β \rightarrow 2)-phloroglucinol and (6) are observed at room temperature, with single sharp signals for each proton or carbon. In contrast the spectra of the 'crossed' 2,3-*cis* dimers (7) and (8) manifested broadening and multiplicity of many ¹³C resonances at 30 °C (Table 1). In particular C-4a and C-8' appear as very broad signals, C-3 is a broad doublet, and C-4 and C-8' are also broad signals. On heating to 50 °C, C-3, C-4, and C-8 sharpen, while C-4a and C-8' remain broad. The rotational isomers had approximately equal populations.

This implied that the barrier to rotation in the 'crossed' phenols (7) and (8) was higher than in the 'normal' phenols (2) and (6). This apparently is a consequence of the 'crossed' stereochemistry resulting in a closer approach by the B-ring of the lower flavanoid unit to the upper flavanoid unit leading to stronger steric interactions.

Both ¹³C n.m.r. spectra of the 2,3-*trans*-phenols (14) and

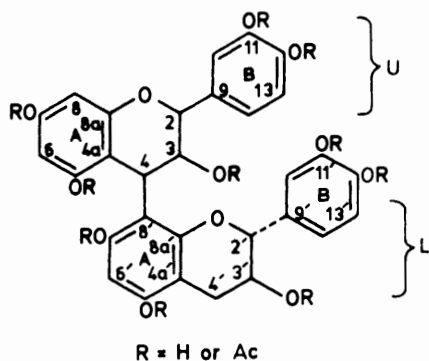


Figure 1. Numbering for procyanidin dimers (this is different from that normally used for flavonoids, but the change was invoked for convenience of discussion)

(15) at 30 °C clearly manifest separate spectra for the two lower energy rotamers, the relative abundance of the two states being 3.5 : 1 for the 'normal' dimer (14) and 1.8 : 1 for the 'crossed' dimer (15). In each case two spectra may be distinguished, one with ^{13}C shifts close to the model compounds catechin-(4 α \rightarrow 2)-phloroglucinol and catechin (Table 1, the minor rotamer in each case), and the other (the major rotamer) which displays small (*ca.* 0.5 p.p.m.) chemical-shift deviations from the spectra of these compounds. Dreiding models show that the 2,3-*trans* (a) rotamer (Figure 2) has the flavanoid units disposed so as to lead to shielding effects on carbons surrounding the interflavanoid bond largely induced by ring currents. The observed chemical-shift deviations are about the same as observed in the ^1H n.m.r. spectra of these compounds ¹ in accordance with theory.²³

(b) *Acetate spectra.* The n.m.r. spectra of the deca-acetate derivatives (18)–(21) of both the 2,3-*cis* and -*trans* dimers manifested the effects of conformational isomerism because of the increased rotational barrier produced by the bulky acetate functions. Simplified spectra may be produced on heating ¹ as illustrated by the ^1H n.m.r. spectra of (20), (21), and epicatechin-(4 β \rightarrow 2)-phloroglucinol octa-acetate at elevated temperature (Table 2). The spectra of the 'normal', (20), and 'crossed', (21), deca-acetates are very similar at high temperature, as would be expected for conformationally averaged molecules.

High-field (360 MHz) ^1H n.m.r. spectra of the deca-acetates (18)–(21) in CDCl_3 enabled a detailed analysis of the spectra to be carried out. The 2,3-*cis* dimers gave readily assigned spectra for each rotational isomer, the relative population of the two states being 3.3 : 1 for the 'normal' dimer (20) and 1.3 : 1 for the 'crossed' dimer (21). Whereas compound (18) had a conformer ratio of 4 : 1 in nitro[$^2\text{H}_5$]benzene,¹ enabling estimation of the free energy of rotation, the same compound exists as predominantly one conformer in CDCl_3 , as does the 'crossed' dimer (19). Low-intensity signals attributable to the minor isomer were observable in each case, and the ratio of conformers was estimated to be *ca.* 20 : 1 and 10 : 1 for (18) and (19) respectively. The signals of the minor isomers were of too low an intensity to make reliable assignments.

As with the phenols, consideration of the ^1H and ^{13}C n.m.r. spectra of the 2,3-*cis*-acetates revealed that one rotamer spectrum corresponded well with those for epicatechin penta-acetate and epicatechin-(4 β \rightarrow 2)-phloroglucinol octa-acetate, and one possessed considerable chemical-shift deviations (see Tables 1 and 2). This enabled the 2,3-*cis* (a) conformer to be assigned to the major and minor rotamers of (20) and (21) respectively, and the 2,3-*cis* (b) conformer to the minor and major rotamers of (20) and (21), respectively.

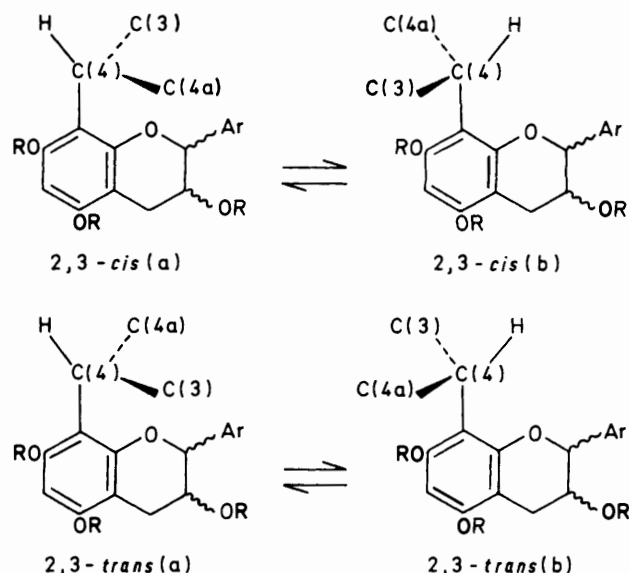


Figure 2. Rotational isomers of dimers containing an upper (U) (2*R*)-procyanidin unit

Consideration of the ^1H and ^{13}C n.m.r. spectra of the 2,3-*trans* dimer acetates shows that as both spectra display considerable (but different) chemical shift deviations from the model compounds catechin penta-acetate and catechin-(4 α \rightarrow 2)-phloroglucinol octa-acetate (see Tables 1 and 2), the predominating rotamers may both be assigned to 2,3-*trans* (a).

Consideration of the ^1H n.m.r. spectra in CDCl_3 of the 2,3-*trans* (a) and 2,3-*cis* (a) conformers of the four dimers (18)–(21) shows that the spectra of the pairs of dimers (18) and (21), and (19) and (20), are qualitatively related. The latter spectra are characterised by increased shielding of the 2'-H, 6-H, and 8-H proton signals, whereas the spectra of (18) and (21) are characterised by very similar chemical shifts for 2'-H, 6-H, and 8-H, which are only slightly shielded relative to the model compounds, and increased shielding of the lower flavanoid unit B-ring 10'-H and 14'-H protons.

These correlations suggest a structural relationship between these pairs of dimers, which becomes evident if the absolute configuration of the chiral centres of each dimer is considered. These are: compound (18) (2*R*,3*S*,4*S*,2'*R*,3'*S*); compound (19) (2*R*,3*S*,4*S*,2'*S*,3'*R*); compound (20) (2*R*,3*R*,4*R*,2'*R*,3'*R*); and compound (21) (2*R*,3*R*,4*R*,2'*S*,3'*S*). If the absolute configurations at C-4 and C-2' are considered it may be seen that the pair of dimers (18) and (21) have a *meso*-relationship with (19) and (20), and each pair has the same *relative* configuration about the interflavanoid bond. The consequence of this is that the relative orientation of the key deshielded protons to the π -electron ring currents in the neighbouring flavanoid unit is the same in each pair, leading to qualitatively similar chemical shifts.

Heterocyclic Ring Conformation.—Earlier studies based on the ^1H n.m.r. of flavans²⁴ and using a simple Karplus²⁵ approach were able to argue strongly for a half-chair conformation for the heterocyclic ring of 2,3-*trans*-flavan-3-ols, with the 2-aryl group pseudo-equatorial. However, the same approach failed to yield an unequivocal choice between half-chair and C(2)-sofa conformations for epicatechin²⁴ (the sofa and half-chair conformations of the pyran ring are shown in Figure 3).

Subsequently *X*-ray diffraction studies of derivatives of

Table 1. ^{13}C N.m.r. chemical shifts

Compound ^a	Chemical shifts (p.p.m.) ^b					
	C-2	C-3	C-4	C-4a	C-6	C-8
(i) 2,3-<i>cis</i>-Flavonoids						
(a) Phenols						
Epicatechin	79.1	66.8	28.6	100.3	96.8	96.0
Epicatechin-(4 β →2)-phloroglucinol	76.6	72.6	36.5	102.0	96.5	95.7
(2) and (6) U	76.6	72.8	36.7	102.2	96.5	96.0
L	79.1	66.2	29.1	100.6	97.4	107.7
(7) and (8) U	76.6	72.5d	36.6b	ca. 102vb	96.0vb	96.4
L	78.8	66.8b	28.8	100.4	97.1	108vb
(8) ^c U	76.6	72.8	36.5	ca. 102vb	96.5b	96.8
L	79.0	66.8	28.9	100.7	97.4	108b
(b) Acetates						
Epicatechin	76.7	66.7	26.1	109.7	108.8	108.0
Epicatechin-(4 β →2)-phloroglucinol	73.5	70.8	33.9	110.5	108.8	107.7
(20) U major	73.7	71.1	34.1	111.7	108.7	107.3
L conformer	77.3	66.8	26.7	111.6	110.4	116.8
U minor	74.4	70.7	34.1	111.7	109.0	108.1
L conformer	76.8	66.4	26.4	110.9	109.8	117.5
(21) U major	74.4	69.9	33.2	111.8	108.8	107.5
L conformer	77.5	66.6	26.7	111.1	109.8	117.4
U minor	74.4	70.3	33.4	111.8	108.8	107.5
L conformer	78.6	66.1	26.2	111.1	110.4	116.6
Epicatechin-(4 β →6) U	73.7	70.9	34.5	111.0	108.7	107.5
Epicatechin L	76.6	66.3	26.4	109.8	116.5d	111.7d
Epicatechin-(4 β →6) U	73.9	71.0d	33.9	111.7	108.7	107.7d
ent-Epicatechin L	76.6	66.6d	26.5d	110.0	116.8d	110.7d
(ii) 2,3-<i>trans</i>-Flavonoids						
(a) Phenols						
Catechin	82.0	67.9	28.1	100.9	96.7	95.7
Catechin-(4 α →2)-phloroglucinol	83.7	72.9	37.9	106.8	97.6	96.4
(14) U Major	83.2	73.5	37.9	107.0	96.7	96.4
L conformer	81.4	68.2	28.1	102.0	97.2	108.6
U Minor	83.7	72.8	37.9	107.0	97.6	96.2
L conformer	82.4	68.2	28.6	100.6	96.7	108.2
(15) U Major	83.1	73.5	38.0	107.0	96.3	96.2
L conformer	82.0	67.8	29.0	102.1	96.3	108.8
U Minor	83.5	72.9	38.0	106.7	97.4	96.2
L conformer	82.0	68.0	27.4	100.0	97.4	107.9
(b) Acetates						
Catechin	77.8	68.4	24.0	110.2	108.8	107.7
Catechin-(4 α →2)-phloroglucinol	79.3	71.6	36.8	114.9	110.4	108.7
(18) U	79.0	70.6	36.7	115.2	109.6	108.3
L	78.2	68.5	25.8	111.6	110.1	117.0
(19) U	79.2	71.6	36.6	115.2	109.5	107.8
L	77.7	68.0	25.0	111.8	109.9	117.3

^a U = Upper, and L = lower flavanoid unit (see Figure 1, also for numbering). ^b All spectra at 20 MHz and 30 °C; phenols in [$^2\text{H}_6$]acetone-H₂O (1 : 1 v/v) and acetates in CDCl₃, chemical shifts relative to internal TMS; b = broad (ca. 10 Hz width at half height); vb = very broad (~20 Hz width at half height, or more); d = doublet. ^c Spectrum at 50 °C.

both catechin²⁶ and epicatechin²⁷ have been completed, together with the closely related *cis*- and *trans*-diphenyl flavan-3-yl carbinols.²⁸ The crystal structures showed that the 2-aryl groups in both catechin and epicatechin are pseudo-equatorial.^{26,27} Both epicatechin²⁷ and the *cis* isomer²⁸ adopt a C(3)-sofa conformation with dihedral angles close to those theoretically predicted from cyclohexene.²⁹ The conformation adopted by the 2,3-*trans* isomers is quite different, with catechin²⁶ adopting a position intermediate between a C(2)-sofa and a half-chair conformation, and the *trans* isomer a position intermediate between a C(3)-sofa and a half-chair.²⁸

An empirical generalisation of the Karplus equation involving six variable parameters has recently been developed.³⁰ This enabled calculation of the theoretical coupling constants

for the 2,3-*cis* and 2,3-*trans* dimers with allowance for the electronegativity of the substituents. Such interactions would be expected to lead to large deviations of J from those predicted by a simple Karplus approach, especially for the 2,3-*cis* series where O-1 and O-3 are nearly diaxially related to 3-H and 2-H respectively, leading to a maximum electronegative effect.³¹

Coupling constants obtained from the empirical Karplus equation are summarised in Table 3, using dihedral angles obtained from the crystallographic ring conformations of epicatechin and catechin,^{26,27} together with values for the theoretical half-chair and sofa conformations.^{28,29}

It may be seen that in general there is excellent agreement between the observed and calculated coupling constants. This

Table 2. ¹H N.m.r. spectra (δ values; J in Hz; SiMe₄ standard) *

Compound	2-H	3-H	4-H	6-H	8-H	OAc	B-Ring
(i) 2,3- <i>cis</i> -Flavanoids							
(a) Phenols							
Epicatechin	4.85	4.20	2.78	5.92	6.00		
Epicatechin-(4 β →2)-phloroglucinol	5.07	4.01	4.55		5.92s		
(2) and (6) U	5.09	4.00	4.95		6.01s		
L	4.73	4.33	2.88	5.97			
(7) and (8) U	5.08	4.26	4.98		6.02s		
L	4.72	4.26	2.87	5.98			
(b) Acetates							
Epicatechin	5.10	5.40	2.92	6.56	6.65	1.90(1), 2.27(4)	
Epicatechin-(4 β →2)-phloroglucinol	5.45s	5.14	4.45	6.59	6.77	1.84(1), 1.88(1), 2.00(1), 2.26(1), 2.34(4)	
Epicatechin-(4 β →2)-phloroglucinol ^a	5.42	5.35	4.57	6.52	6.66		
(20) ^b U	5.56	5.45	4.65	6.33	6.39		
L	5.29	4.70	2.95	6.68			
(21) ^c U	5.60	5.43	4.67		6.36s		
L	5.36	4.67	2.93	6.68			
(20) ^d U Major	5.58	5.16	4.46	5.99	6.24	1.60(1), 1.88(2), 1.99(1), 2.04(1)	10'-H, 7.01; 13'-H, 7.04
L conformer	4.54	5.10	2.86, 2.94	6.66		2.19(1), 2.30(3), 2.37(1)	14'-H, 6.89; rest 7.1—7.4
U Minor	5.41	5.33	4.64	6.63	6.77	1.56(1), 1.74(1), 1.86(1), 1.88(1)	7.16—7.37
L conformer	5.26	5.55	2.98, 3.08	6.58		2.03(1), 2.26(2), 2.27(2), 2.31(1)	
(21) ^d U Major	5.49	5.53	4.66	6.66	6.73	1.58(1), 1.83(1), 1.85(1), 1.88(1), 1.99(1)	7.12—7.38
L conformer	5.03	5.37	2.92, 3.11	6.62		2.25(1), 2.28(1), 2.30(2), 2.31(1)	
U Minor	5.33	5.34	4.46	6.43	6.59	1.58(2), 1.85(1), 1.89(1), 1.93(1)	10'-H, 6.76; 13'-H, 7.07
L conformer	4.91	5.11	2.84, 2.94	6.68		2.24(1), 2.28(2), 2.30(1), 2.31(1)	14'-H, 6.69; rest 7.1—7.4
(ii) 2,3- <i>trans</i> -Flavanoids acetates							
Catechin	5.15	5.15	2.68, 2.79	6.49	6.67	1.95(1), 2.22(4)	
Catechin-(4 α →2)-phloroglucinol	4.88	5.67	4.58	6.49	6.67	1.64(1), 1.92(1), 1.95(1)	
(18) ^d U	4.76	5.63	4.48	6.48	6.51	2.21(1), 2.24(3), 2.32(1)	10-H, 7.02; 13-H, 7.14; 14-H, 6.99
L	4.96	5.02	2.66, 2.93	6.65		1.66(1), 1.75(1), 1.99(1), 2.25(1)	10'-H, 6.93; 13'-H, 7.13; 14'-H, 6.72
(19) ^d U	4.76	5.65	4.55	6.26	6.38	1.57(1), 1.68(1), 1.92(1), 1.93(1)	7.14—7.25
L	4.60	5.16	2.53, 2.98	6.61		2.23(1), 2.26(1), 2.29(3), 2.36(1)	

* All spectra run at 30 °C and 80 MHz, phenols in [²H₆]acetone and acetates in CDCl₃ unless stated otherwise. ^a At 120 °C in [²H₅]nitrobenzene. ^b At 180 °C in [²H₅]nitrobenzene. ^c At 160 °C in [²H₅]nitrobenzene. ^d At 360 MHz.

particularly applies to the epicatechin series where agreement between calculated and observed values using a simple Karplus approach were very poor, and encouraged the view that the effects may be due to conformational lability in the heterocyclic ring.²⁴ However, it may be seen that the values for epicatechin and its 4-*C*-aryl derivative are clearly consistent with a C(3)-sofa conformation.

The issue is not so clear cut for the catechin series. Theoretical values for the most likely conformations, the crystallographic, and theoretical half-chair and C(2)-sofa conformations are all very similar. This is because in all these conformations 2-H and 3-H have a pseudo-axial relationship, and quite large

differences in dihedral angle ($\pm 10^\circ$) make little difference to the coupling constants. However, a near half-chair conformation appears to agree best with the preferred geometry. The only coupling constant which is seriously in error is $J_{3,4ax}$ for catechin, which is *ca.* 3 Hz smaller than predicted. This suggests that some distortion of its pseudo-axial orientation may have occurred, which receives some support from the fact that $J_{3,4ax}$ in the lower unit of (18) and (19) approaches the theoretical value more closely, while $J_{2,3}$ and $J_{3,4eq}$ remain unchanged.

¹H N.m.r. spectra of (18) and (19) displayed identical coupling constants, which implied that the heterocyclic ring



Figure 3. Heterocyclic ring conformations. Solid lines indicate bonds above, and dashed lines bonds below the plane of the benzene ring

conformation remains unchanged and both approximate to half-chairs.

In contrast, the ^1H n.m.r. of the 2,3-*cis* dimer deca-acetates, (20) and (21), indicated that a change in heterocyclic ring conformation had occurred in both the upper and lower units in the minor isomer of (21). The coupling constants for this rotamer indicate that the upper unit adopts a twisted C(3)-sofa with C-2 well below the A-ring plane, whereas the other rotamers adopt a near half-chair conformation with $J_{3,4eq} < 2$ Hz.

The lower unit of the minor rotamer of (21) adopts a half-chair conformation, whereas $J_{3,4eq}$ for the other rotamers is *ca.* zero, indicating a conformation approximating to a C(2)-sofa.

Conclusions

The (a)-type conformation is considerably favoured in the 2,3-*trans* dimer deca-acetates, whereas the situation is equivocal for the 2,3-*cis* dimers. The effect of adopting an (a)-type conformation is to minimise the surface area of the molecule, and hence solute-solvent contact. Two effects counteract the adoption of an (a)-type conformation: (i) the gain in stability by dipole-induced dipole interactions between chloroform and the aromatic rings (an enthalpy gain of 1–2 kcal mol $^{-1}$ *),³¹ and (ii) steric interactions between the flavanoid units which are maximum in this conformation. Stabilisation of the (a)-type conformation apparently arises by minimisation of the disruption of solvent-solvent interactions. The cohesive energy of chloroform is estimated³³ to be 7 kcal mol $^{-1}$ or just over 1 kcal mol $^{-1}$ per interacting pair of solvent molecules, assuming that each chloroform has a co-ordination sphere of six.

The free-energy difference between the low-energy states of rotational isomers is small. A population ratio of 10:1 is equivalent to a free-energy difference of *ca.* 1 kcal mol $^{-1}$.³⁴ Therefore the energy difference is the same order of magnitude as the dipole-induced dipole solute-solvent, and solvent-solvent interactions. It may be assumed that these latter interactions are about the same for all the (a)-type conformers, so that the (a):(b) population is largely determined by steric interactions, which will destabilize (a) relative to (b). Dreiding models show that these are least severe for the (a)-conformers of the 2,3-*trans* dimers, and the ratio of (a):(b) is high. In contrast the models show that as the C(4)–C(8)' bond is very nearly normal to the plane of the U-unit A-ring in the 2,3-*cis* dimers, O-1', C-2', and C-9' are in a plane approximately parallel to the U-unit, A-, and heterocyclic rings, resulting in strong mutual steric interactions between the units. These are most severe for the 'crossed' 2,3-*cis* dimer as the opposing absolute configurations at C-2 and C-2' result in the B-rings being held in closer proximity to one another than in the 'normal' dimer, leading to stronger steric interactions.

Table 3. Experimental and theoretical $^3J_{\text{HH}}$ coupling constants (Hz) for flavanoid acetate derivatives

Flavanoid unit	$J_{2,3}$	$J_{3,4ax}$	$J_{3,4eq}$
2,3- <i>cis</i> -OC unit (observed)	1.1		2.5
Theoretical half-chair	0.5		1.7
Theoretical C(3)-sofa	1.2		2.2
2,3- <i>trans</i> -PC unit (observed)	10.0	9.4	
Theoretical 'crystallographic'	9.1	9.0	
Theoretical half-chair	9.1	9.4	
C(2)-sofa	9.2	11.0	
Epicatechin (observed)	1.0	2.9 (average)	
Theoretical half-chair	0.5	4.0	2.1
Theoretical C(2)-sofa	0.8	6.8	0
Theoretical C(3)-sofa	1.2	2.5	2.7
(20), Major conformer, L	<i>ca.</i> 0	5.4	<i>ca.</i> 0
(20), Minor conformer, L	<i>ca.</i> 0	4.3	<i>ca.</i> 0
(21), Major conformer, L	<i>ca.</i> 0	5.0	<i>ca.</i> 0
(21), Minor conformer, L	<i>ca.</i> 0	4.0	2.2
Catechin (observed)	8.5	7.0	5.5
Theoretical 'crystallographic'	9.1	9.7	6.1
Theoretical half-chair	8.5	10.5	5.5
Theoretical C(2)-sofa	8.9	10.0	5.6
(18) and (19)	8.5	8.3	5.8

These interactions are apparently strong enough to result in changes in the conformation of the heterocyclic rings of both units of the 'crossed' dimer (21).

Experimental

^1H N.m.r. spectra were recorded at 80 MHz and ^{13}C n.m.r. at 20 MHz with a Varian FT-80A spectrometer. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter, and circular dichroism measurements on a Jasco Model ORD/UV-5 in methanol. H.p.l.c. was carried out on Waters μ Bondapak C-18 columns using methanol-water-acetic acid (20:79:1 v/v) as solvent, using a flow rate of 2.0 ml/min.

Cellulose t.l.c. analyses were performed on Schleicher and Schull F1440 sheets using 6% acetic acid as the developing solvent. Procyanidins were visualised with a 5% vanillin in ethanol-concentrated hydrochloric acid (5:1, v/v) spray reagent.

ent-Epicatechin (1).—Catechin (4.0 g) was dissolved in ethanol (25 ml), placed in a glass-lined stainless steel bomb, flushed twice with nitrogen, pressurised to 2 000 lb in $^{-2}$ under nitrogen and placed in a preheated oven at 180 °C for 2 h. The product was chromatographed on Sephadex LH-20, solvent ethanol-water (15:85 v/v), and the eluant collected as 20-ml fractions, and monitored by t.l.c., to yield initially pure *ent*-epicatechin (0.63 g), which was recrystallised from water to yield colourless rods, m.p. 235–237 °C (lit.,³⁵ m.p. 234–238 °C), $[\alpha]_{578}^{30} + 56.5^\circ$ (*c* 0.20 in acetone-water, 1:1) [lit. value, $[\alpha]_{589}^{30} + 59.0^\circ$ (*c* 2.0 in acetone-water, 1:1)], followed by catechin. *ent*-Catechin (13) was prepared as for *ent*-epicatechin, or epicatechin (10 g) was dissolved in 5% sodium hydroxide, previously heated to 65 °C, and left to stand for 10 min. The solution was immediately cooled in an ice-bath, acidified with 2M-hydrochloric acid, and the resulting solution extracted with ethyl acetate (3 \times 100 ml). Concentration of the dried (Na $_2$ SO $_4$) ethyl acetate solution gave a brown solid which was chromatographed on Sephadex LH-20 in ethanol-water (15:85) to yield epicatechin in the initial fractions, followed by *ent*-catechin (2.3 g) which was recrystallised from water to

* 1 Cal is 4.184 J.

yield colourless needles, m.p. 175–176 °C (lit.,³⁵ m.p. 173–175 °C), $[\alpha]_{578}^{30} - 17.4^\circ$ (c 2.3 in acetone–water, 1 : 1 v/v) {lit. value, $[\alpha]_{589}^{20} - 16.0^\circ$ (c 2.0 in acetone–water, 1 : 1 v/v)}.

ent-Epicatechin-(4 α \rightarrow 8)-*epicatechin* (7).—Palm procyanidin polymer (3.0 g) and epicatechin (3.0 g) were dissolved in ethanol (15 ml), acetic acid (0.3 ml) added, and the solution heated in a sealed vial under nitrogen at 95 °C for 22 h. The cooled solution was subsequently evaporated under reduced pressure and the resulting residue dissolved in water (50 ml) and extracted with ethyl acetate (5 \times 50 ml). The combined ethyl acetate solutions were dried (Na₂SO₄) and evaporated to yield a brown solid (5.7 g) which was chromatographed on Sephadex LH-20 in ethanol to yield fractions containing 4 \rightarrow 8 linked dimers, R_F 0.65. These were combined and evaporated under reduced pressure to yield a fawn solid (0.85 g) which was further fractionated by h.p.l.c. to yield (7), R_v 8.4 ml, obtained, after freeze-drying, as a white *solid* (Found: C, 61.1; H, 5.0. C₃₀H₂₆O₁₂·0.5H₂O requires C, 61.3; H, 4.6%), $[\alpha]_{578}^{30} - 182^\circ$ (c 0.15 in CH₃OH–H₂O, 1 : 1 v/v).

The *deca-acetate* (acetic anhydride–pyridine) of (7) was purified by t.l.c. [benzene–acetone (8 : 2 v/v); silica; R_F 0.28] to yield a white amorphous *solid* (Found: C, 59.5; H, 5.0. C₅₀H₄₆O₂₂·0.5H₂O requires C, 59.6; H, 4.7%); $[\alpha]_{578}^{30} - 131^\circ$ (c 0.67 in acetone); c.d. maxima $\Delta\epsilon_{230} - 3.66$, $\Delta\epsilon_{265} - 0.65$.

Epicatechin-(4 β \rightarrow 8)-epicatechin (6) was separated from (7) by h.p.l.c., R_v 10.0 ml, $[\alpha]_{578}^{30} + 24.5^\circ$ (c 0.20 in CH₃OH–H₂O, 1 : 1 v/v) {lit.,¹² $[\alpha]_{578}^{20} + 15.2^\circ$ (c 1.2 in EtOH)}. The *deca-acetate* was prepared as for (7), $[\alpha]_{578}^{30} + 52.0^\circ$ (c 0.63 in acetone) {lit.¹² $[\alpha]_{578}^{20} + 54^\circ$ (c 1.4 in acetone)}; c.d. maxima $\Delta\epsilon_{230} + 3.22$, $\Delta\epsilon_{250} + 0.51$.

ent-Epicatechin-(4 α \rightarrow 8)-*ent-epicatechin* (2), was prepared from the palm procyanidin polymer (1.6 g) by reaction with *ent-epicatechin* (1.6 g) in a sealed tube as described previously. Chromatography of the reaction product on Sephadex LH-20 gave fractions containing 4 \rightarrow 8 linked dimers, crude yield 0.22 g. Further chromatography (h.p.l.c.) separated the diastereoisomeric dimer (8), R_v 8.4 ml, from (2), R_v 10.0 ml, which was obtained after freeze-drying as a white *solid* (Found: C, 61.4; H, 4.8. C₃₀H₂₆O₁₂·0.5H₂O requires C, 61.3; H, 4.6%), $[\alpha]_{578}^{30} - 23.9^\circ$ (c 0.20 in CH₃OH–H₂O, 1 : 1 v/v).

The *deca-acetate*, R_F 0.40 [benzene–acetone (8 : 2 v/v); silica t.l.c.] was obtained as a white amorphous *solid* (Found: C, 59.6; H, 4.8. C₅₀H₄₆O₂₂·0.5H₂O requires C, 59.6; H, 4.7%); $[\alpha]_{578}^{30} - 43^\circ$ (c 0.16 in acetone), c.d. maxima $\Delta\epsilon_{230} - 3.41$, $\Delta\epsilon_{275} - 0.38$.

Epicatechin-(4 β \rightarrow 8)-*ent-epicatechin* (8) was prepared by reaction between the procyanidin polymer from *Aesculus carnea* (fruit) (2.0 g) and *ent-epicatechin* (2.0 g) as described previously. The reaction product (0.26 g) was chromatographed on Sephadex LH-20, and the crude dimer purified by h.p.l.c. to yield (8), R_v 8.4 ml, as a white *solid* $[\alpha]_{578}^{30} + 187^\circ$ (c 0.24 in CH₃OH–H₂O, 1 : 1 v/v).

The *deca-acetate*, R_F 0.30 [benzene–acetone (8 : 2 v/v); silica t.l.c.] was obtained as a white amorphous *solid* (Found: C, 59.6; H, 4.8. C₅₀H₄₆O₂₂·0.5H₂O requires C, 59.6; H, 4.7%); $[\alpha]_{578}^{30} + 134^\circ$ (c 0.54 in acetone), c.d. maxima $\Delta\epsilon_{230} + 3.76$, $\Delta\epsilon_{270} + 0.65$.

Epicatechin-(4 β \rightarrow 6)-*ent-epicatechin* (9).—Later fractions from the LH-20 separation of (8) were shown (cellulose t.l.c.) to contain a mixture of the 4 \rightarrow 8 (R_F 0.65) and 4 \rightarrow 6 (R_F 0.35) linked dimers. The mixture was acetylated and the products separated by t.l.c. [benzene–acetone (8 : 2 v/v); silica] to yield the *deca-acetates* of (8) (40 mg), R_F 0.30, and (9) as an amorphous *solid* (80 mg) (Found: C, 60.0; H, 4.8.

C₅₀H₄₆O₂₂ requires C, 60.1; H, 4.6%), R_F 0.40, $[\alpha]_{578}^{30} + 77^\circ$ (c 0.16 in acetone).

Catechin-(4 α \rightarrow 8)-*ent-catechin* (15).—Taxifolin (2.0 g) and sodium borohydride (1.0 g) were stirred in absolute ethanol (200 ml) for 1 h at room temperature, *ent-catechin* (1.8 g) was dissolved in the reaction mixture, and 200 ml of 0.1M-hydrochloric acid added. The reactants were stirred for 1 h, ethanol was removed under reduced pressure at 40 °C, and the aqueous residue extracted with ethyl acetate (5 \times 100 ml). The combined ethyl acetate extract was dried (Na₂SO₄) and evaporated to yield a solid (3.2 g) which was chromatographed on Sephadex LH-20 eluted with ethanol. Unchanged *ent-catechin* was eluted, followed by the dimer (15), crude yield 0.50 g, R_F 0.50 (t.l.c., cellulose), and was purified by h.p.l.c., R_v 7.6 ml, to yield a white *solid* (Found: C, 62.7; H, 5.0. C₃₀H₂₆O₁₂ requires C, 62.3; H, 4.5%), $[\alpha]_{578}^{30} - 234^\circ$ (c 0.26 in CH₃OH–H₂O, 1 : 1 v/v).

The *deca-acetate* of (15), R_F 0.50 [benzene–acetone (8 : 2); silica, t.l.c.], was obtained from methanol as colourless *needles* (Found: C, 60.3; H, 5.1. C₅₀H₄₆O₂₂ requires C, 60.1; H, 4.6%), m.p. 134–137 °C, $[\alpha]_{578}^{30} - 144^\circ$ (c 0.31 in acetone), c.d. maxima: $\Delta\epsilon_{232} - 3.13$, $\Delta\epsilon_{275} - 1.72$, $\Delta\epsilon_{290} - 0.52$.

Catechin-(4 α \rightarrow 8)-*catechin* (14).—Similar reaction between reduced taxifolin and catechin yielded the phenol (14), purified by h.p.l.c., R_v 7.0 ml, $[\alpha]_{578}^{30} - 235^\circ$ (c 0.31 in CH₃OH–H₂O, 1 : 1 v/v).

The *deca-acetate* of (14) had $[\alpha]_{578}^{30} - 134^\circ$ (c 0.91 in acetone) {lit.,¹² $[\alpha]_{578}^{30} - 134^\circ$ (c 1 acetone)}, c.d. maxima: $\Delta\epsilon_{230} - 3.95$, $\Delta\epsilon_{274} - 1.91$, $\Delta\epsilon_{295} - 0.41$.

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