Conformations of Polymeric Proanthocyanidins composed of (+)-Catechin or (−)-Epicatechin joined by 4 → 6 Interflavan Bonds

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The conformations of homopolymers of (+)-catechin and (−)-epicatechin, linked from C(4) of one monomer to C(6) to the next, have been established by the combination of the results from recent MM2 calculations and time-resolved fluorescence studies. Matrix methods were used for evaluation of the unperturbed dimensions and persistence vectors. The polymers linked from C(4) to C(6) tend to be more compact than polymers linked from C(4) to C(8).

Condensed tannins are polymers of (+)-catechin and (−)-epicatechin. Structures of the monomers are depicted in Figure 1. The most common linkage in naturally occurring condensed tannins is from C(4) of one monomer to C(8) of its neighbour.[1,2] There are two rotational isomers at this interflavan bond.[3–7] The conformation of a polymer molecule would be a helix if one rotational isomer were populated to the exclusion of the other.[8,9] Recent time-resolved fluorescence measurements have shown that both rotational isomers are populated in epicatechin-(4β → 8)-catechin, with the ratio of the populations being *ca.* 3:1.[3,4,7] The population of both rotational isomers by the dimer implies that 4 → 8 linked polymers are not helical rods, but instead are random coils with unperturbed dimensions not too different from those seen in more familiar polymers such as poly(styrene).[10] This conclusion is compatible with the behaviour of the peracetylated polymers and poly(styrene) upon gel permeation chromatography in tetrahydrofuran.[11]

Another type of linkage, from C(4) of one monomer to C(6) of its neighbour, is also found in naturally occurring condensed tannins.[12–14] Conformational properties of the 4 → 6 linked polymers are described here. Attention is given to the influences of the type of monomer, the stereochemistry of the attachment of the interflavan bond at C(4), and the population of the two rotational isomers at the interflavan bond. Of particular interest are the conformational properties expected when the population of the rotational isomers is that deduced from the recent time-resolved fluorescence study of epicatechin-(4β → 6)-catechin.[3,4,7]

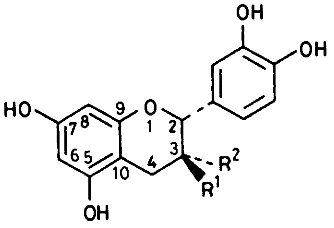

Figure 1. Structure of (+)-catechin (R^1 = OH, R^2 = H) and (−)-epicatechin (R^1 = H, R^2 = OH)

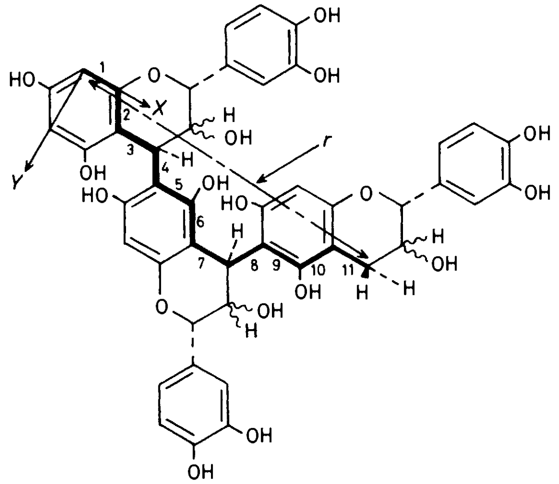

Figure 2. Definition of the main chain (thick bonds) for a proanthocyanidin trimer with 4 → 6 interflavan bonds. The end-to-end distance is denoted by *r*, and the *X* and *Y* axes of the internal co-ordinate system used for expression of the end-to-end vector are shown

Results and Discussion

Definition of the Main Chain.—Figure 2 depicts a trimer in which the monomers are connected by two 4 → 6 interflavan bonds. The main chain is defined as the one that begins at C(8) of the monomer with an unbonded C(6) and proceeds to the unbonded C(4). The bonds in the main chain are drawn with thick lines in Figure 2. The number of such bonds is $4x - 1$, where x denotes the number of monomer units in the polymer. The bonds are indexed from 1 to n, with $n = 4x - 1$.

The main chain is defined so that it begins at C(8), rather than C(6), in order to facilitate comparison with the persistence vectors of the 4 → 8 linked polymers considered earlier.[10] The persistence vectors are expressed in an internal co-ordinate system defined by the first two bonds. The X axis of this co-ordinate system is parallel to bond 1, and the Y axis is in the plane of bonds 1 and 2, with a positive projection on bond 2. These two axes are depicted for 4 → 6 linked polymers in Figure 2, and for 4 → 8 linked polymers in Figure 3. The Z axis completes a right-handed co-ordinate system. When the main chain is drawn in the manner employed in Figure 2, this internal co-ordinate system is identical with the one used for the description of the 4 → 8 linked polymers.[10] Therefore vectors expressed in the internal co-ordinate systems for 4 → 6 and 4 → 8 linked polymers may be compared without the necessity for transforming from one co-ordinate system to another.

Unperturbed Dimensions.—The end-to-end distance of the main chain is denoted by r, as shown in Figure 2. The mean square unperturbed end-to-end distance, denoted $\langle r^2\rangle_0$, was obtained using the matrix methods described by Flory.[15] Details can be found in the Calculations section. The mean square unperturbed end-to-end distance for a chain of x monomer units can be converted into a dimensionless characteristic ratio defined as $\langle r^2\rangle_0/\Sigma l_i^2$, where Σl_i^2 is the sum

Table 1. Values of C for different fractions of the positive rotamer

Monomer	Linkage	f 0.17	0.50	0.60	0.83
(+)-Catechin	$4\alpha \longrightarrow 6$	8.3	2.9	3.0	5.7
(−)-Epicatechin	$4\alpha \longrightarrow 6$	9.6	3.2	3.0	5.3
(+)-Catechin	$4\beta \longrightarrow 6$	14.7	4.1	3.8	5.0
(−)-Epicatechin	$4\beta \longrightarrow 6$	12.3	4.3	3.9	5.8

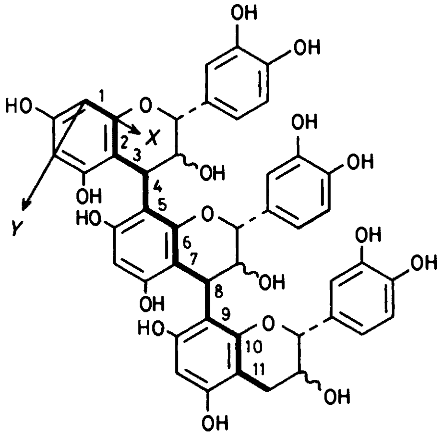

Figure 3. A proanthocyanidin trimer with $4 \longrightarrow 8$ interflavan bonds. The main chain and internal co-ordinate system are those used by Viswanadhan *et al.*[10]

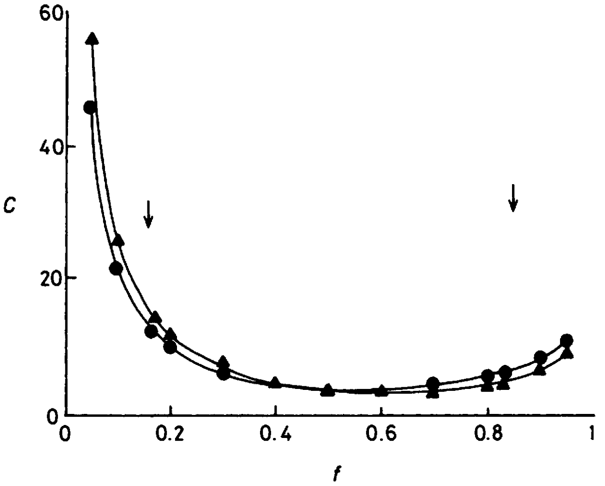

Figure 4. Characteristic ratio at infinite chain length *versus* f for two $4\beta \longrightarrow 6$ linked homopolymers of catechin (triangles) and epicatechin (circles). Arrows are drawn at f 0.15 and 0.85

of the squared lengths of all bonds in the main chain. For any flexible chain, the characteristic ratio approaches an asymptotic limit, denoted by C, as x becomes infinite.

Flexibility is present in the polymeric proanthocyanidins if both rotational isomers at the interflavan bond are accessible. The fraction of the interflavan bonds that occupy the positive rotational isomer will be denoted by f. The positive rotational isomer at bond 4 is obtained by a positive rotation about the interflavan bond, starting from a *cis*-conformation for bonds 3—5 in Figure 2. The other rotational isomer, denoted as being negative, has a dihedral angle displaced nearly 180° from the positive isomer.

Table 2. Parameters for the helices

Monomer	Linkage	f 0 M/Tn	f 0 Trans/M	f 0 Pitch	f 1 M/Tn	f 1 Trans/M	f 1 Pitch
(+)-Catechin	$4\alpha \longrightarrow 6$	−2.9	3.51	10.2	2.4	2.83	6.8
(−)-Epicatechin	$4\alpha \longrightarrow 6$	−2.9	3.72	10.8	2.5	2.69	6.7
(+)-Catechin	$4\beta \longrightarrow 6$	−2.2	3.39	7.5	4.0	3.40	13.6
(−)-Epicatechin	$4\beta \longrightarrow 6$	−2.2	3.16	7.0	3.8	3.60	13.7

M/Tn is the number of monomers per turn, Trans/M is the translation per monomer in Å, and the Pitch is also in Å. Left- and right-handed helices are denoted by negative and positive M/Tn, respectively.

Table 1 and Figure 4 present the manner in which C depends on f for homopolymers of (+)-catechin or (−)-epicatechin with $4 \longrightarrow 6$ interflavan bonds. The smallest C are in the range 2.9—3.9, and they are obtained with f 0.5—0.6. The smallest C for the $4 \longrightarrow 8$ linked polymers are also obtained for f near 0.5, but these values of C are much larger than 4.[10] The smallest C for $4 \longrightarrow 8$ linked polymers is 8.9. The difference in the dimensions of $4 \longrightarrow 6$ and $4 \longrightarrow 8$ linked polymers at f 0.5 is easily rationalized. Successive interflavan bonds, such as bonds 4 and 8 in Figure 2, are more nearly parallel in $4 \longrightarrow 8$ than in $4 \longrightarrow 6$ linked polymers, as can be seen by comparison of Figures 2 and 3. Therefore the $4 \longrightarrow 8$ linked polymers form the more extended random coils.

The data in Table 1 show how the three variables (repeat unit, α or β interflavan linkage, and f) alter the unperturbed dimensions of the homopolymers. The unperturbed dimensions are most sensitive to f. The type of repeat unit, (+)-catechin or (−)-epicatechin, has very little effect on the unperturbed dimensions of the homopolymer. The selection of α or β stereochemistry at C(4) plays an intermediate role.

Recent fluorescence measurements[4,7] ascribed the heterogeneity of the fluorescence decay of epicatechin-($4\beta \longrightarrow 6$)-catechin in dioxane to the presence of two rotational isomers. The relative amount of each rotational isomer was determined by the values of the pre-exponential factors for the bi-exponential decay. The fractional contribution of the major and minor lifetimes were found to be 0.85 ± 0.08 and 0.15 ± 0.08, respectively. The fluorescence measurement does not identify which rotational isomer has the higher population. Since the repeat unit, (+)-catechin or (−)-epicatechin, makes only a small difference in the unperturbed dimensions of the polymer chain, as shown in Table 1, fluorescence measurements for epicatechin-($4\beta \longrightarrow 6$)-catechin would predict that a polymer chain that is linked $4\beta \longrightarrow 6$ should have fractional contribution of *ca.* 0.85 or 0.15 from the (+)-rotamer. The arrows in Figure 4 denote the two values of f that are consistent with the time-resolved fluorescence measurements. If the chain were to have a characteristic ratio of 27, the peracetylated proanthocyanidin polymer would have the same unperturbed dimensions as a poly(styrene) chain of the same molecular weight.[10] The combination of Figure 4 and the time-resolved fluorescence measurements shows that the peracetylated proanthocyanidin polymer should actually be more compact than poly(styrene), as expected from the behaviour upon gel permeation chromatography.[11]

Helices.—The conformation of the polymer becomes a helix in the limit where f approaches either 0 or 1. The translation per monomer unit, number of monomers per turn, and the handedness of each helix are presented in Table 2. The handedness of the helix is determined completely by the

Table 3. Components (in Å) of the end-to-end vector*

Linkage	Monomer	f	$\langle X \rangle$	$\langle Y \rangle$	$\langle Z \rangle$
$4\alpha \longrightarrow 6$	(+)-Catechin	0	158.86	44.44	−60.12
		0.17	11.22	5.70	−1.78
		0.50	4.93	3.22	2.03
		0.83	6.61	0.20	7.67
		1	75.26	−49.51	108.02
$4\alpha \longrightarrow 6$	(−)-Epicatechin	0	176.02	40.80	−44.64
		0.17	12.42	5.60	−1.15
		0.50	5.09	3.25	2.13
		0.83	6.12	0.44	7.62
		1	67.41	−47.95	104.75
$4\beta \longrightarrow 6$	(+)-Catechin	0	87.20	−66.19	−127.34
		0.17	11.49	−3.46	−15.06
		0.50	5.38	2.80	−3.99
		0.83	7.49	5.29	−0.67
		1	157.26	52.92	38.16
$4\beta \longrightarrow 6$	(−)-Epicatechin	0	78.51	−74.89	−112.92
		0.17	10.62	−3.93	−13.18
		0.50	5.72	2.44	−3.87
		0.83	8.56	4.79	−0.99
		1	171.25	41.70	37.27

* As x approaches ∞ for $0 < f < 1$, at $x = 50$ for $f = 0$ or 1

Table 4. Geometry used in the rotational isomeric state model for $4 \longrightarrow 6$ linked polymers

Bond	Monomer	Linkage	Length (Å)	θ (°)	φ (°)
1	Either	Either	1.40	62	
2	Catechin	Either	1.40	60	−179
	Epicatechin	Either	1.40	61	180
3	Either	α	1.52	66	−133
	Either	β	1.51	64	+110
4	Catechin	α	1.53	60,60	40,−138
	Epicatechin	α	1.53	60,68	36,−146
	Catechin	β	1.53	56,62	150,−30
	Epicatechin	β	1.53	56,62	160,−24
5	Either	Either	1.41	60	179
6	Either	Either	1.41	61	−179
7	Catechin	α	1.51	69	42
	Epicatechin	α	1.51	68	40
	Catechin	β	1.51	68	−78
	Epicatechin	β	1.51	72	−77

Dihedral angles are in the convention with $\varphi = 0°$ for a *cis* placement. Two θ and φ are given for the interflavan bond. The first θ and φ are for the positive rotamer. Bonds are numbered in the manner depicted in Figure 2.

selection of the rotational isomer, as was also the case with polymers containing $4 \longrightarrow 8$ interflavan bonds. Helices formed by $4 \longrightarrow 6$ linked polymers tend to be more compact than those formed by $4 \longrightarrow 8$ linked polymers, although the ranges overlap. The translation per monomer for the $4 \longrightarrow 6$ linked helices in Table 2 ranges from 2.69 to 3.72 Å. with an average of 3.29 Å. In contrast, the range for the helices formed by $4 \longrightarrow 8$ linked polymers is 3.10—4.85 Å, with an average of 3.96 Å. In both types of polymer, the mere substitution of one monomer for the other has very little affect on the geometry of the helix.

Persistence Vectors.—The entries for f 0 and 1 in Table 3 show the direction of propagation of the helices, as expressed in the internal co-ordinate system defined by the first two bonds. The X component of the remote end of a long helix is always positive, the sign of the Z component depends solely on f, and the sign of the Y component depends jointly on f and the selection of α- or β-stereochemistry for the interflavan bonds.

The average value of end-to-end vector for an infinitely long flexible chain is commonly named the persistence vector. Components of the persistence vectors at f 0.17, 0.50, and 0.83 are collected in Table 3. Persistence vectors at f 0.50 are easily rationalized with the aid of Figure 2. The remote end of bond 4 must have positive X and Y components, with the X component being the larger. The sign of the Z component will be determined by the stereochemistry at C(4) of the first monomer unit in the chain, with $Z > 0$ for an α link and $Z < 0$ for a β link. If the chain were completely disordered after bond 4, so that, on average, there was no further propagation of the end-to-end vector, the components of the persistence vector would be identical with the components of the remote end of bond 4. This simple description provides a good approximation to the persistence vectors in Table 3 when f 0.5.

Calculations

The bond lengths, bond angles, and dihedral angles were taken from structures of the dimers that were optimized[5] using MM2.[16] The dihydroxyphenyl substituents at C(2) were maintained in a pseudoequatorial position.[5] The parameters used are collected in Table 4. The end-to-end vector for a chain of x monomers was calculated from equations (1)—(4) where

$$\langle \mathbf{r}_x \rangle = \mathbf{A}_{[1}\mathbf{A}_2\mathbf{A}_3(\mathbf{F}_4\mathbf{F}_5\mathbf{A}_6\mathbf{A}_7)^{(x-2)}\mathbf{F}_4\mathbf{F}_5\mathbf{A}_6\mathbf{A}_{n]} \quad (1)$$

$$\mathbf{F}_4 = [f\mathbf{A}_4{}^{+}(1-f)\mathbf{A}_4{}^{-}] \quad (2)$$

$$\mathbf{F}_5 = \mathrm{col}\,(\mathbf{A}_5{}^{+}, \mathbf{A}_5{}^{-}) \quad (3)$$

$$\mathbf{A}_i = \begin{bmatrix} \mathbf{T} & \mathbf{l} \\ \mathbf{0} & 1 \end{bmatrix}_i \quad (4)$$

$\mathbf{A}_{[1}$ and $\mathbf{A}_{n]}$ are the top three rows and last column, respectively, of $\mathbf{A}_i$.[15] The mean square unperturbed dimensions were computed with the substitution of the usual 5×5 generator matrix for $\mathbf{A}_i$, the first row of this matrix being used for $\mathbf{A}_{[1}$.[15]

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