Hindered Rotation and Helical Structures in Natural Procyanidins

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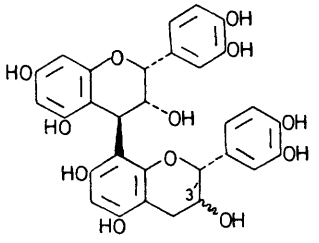
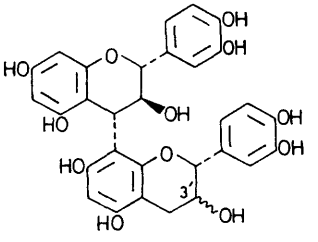
Summary Conformational isomerism has been demonstrated for the four major naturally occurring procyanidins, and the phenomena have been interpreted in terms of two different forms of restricted rotation about the interflavan bond; based on these observations structures of opposite helicity are proposed for the two principal types of procyanidin polymer found in Nature.

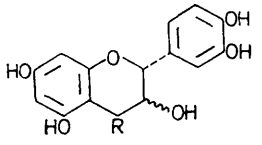
PLANTS yield four principal dimeric procyanidins (**1**)—(**4**) (formulated as C-4 to C-8 linked dimers)[1,2] and biosynthetic studies[3,4] show that these are formed by pathways which parallel the *in vitro* reaction between the carbocation (**7**) or (**8**) and (+)-catechin (**6**) or (−)-epicatechin (**5**). Observations made during the structural investigations[1,5,6,7] suggested that the anomalous n.m.r. spectroscopic behaviour of many of these dimeric structures and their derivatives was due to one or more forms of conformational isomerism. These properties have now been fully explored.



(1), B-1, 3′β-OH
(2), B-2, 3′α-OH



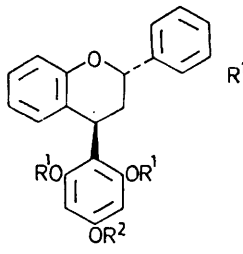
(3), B-3, 3′β-OH
(4), B-4, 3′α-OH

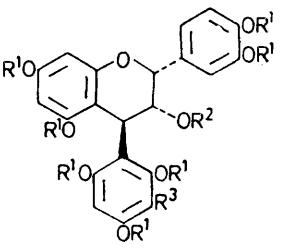


(5), 3α-OH, R=H
(6), 3β-OH, R=H
(7), 3α-OH, R=+
(8), 3β-OH, R=+

Reaction of flavan-4α-ol[8] with phloroglucinol in acidic ethanol gave, as the major products, the adducts (**9**) and (**10**) (one stereoisomeric form depicted). ^{1}H N.m.r. analysis of the methyl ether and acetate derivatives were consistent with a 2,4-*trans*-disposition of the two aryl groups and the adoption by the heterocyclic ring of a *quasi*-chair conformation.[9] Although the phenols (**9**) and (**10**) showed no evidence of conformational isomerism, both the methyl ether and acetate derivatives showed ^{1}H n.m.r. behaviour only explicable in terms of the existence of two conformational forms. Thus, for example, in the acetate (**11**) three acetate methyl signals (δ 1·25, 1·64, and 1·86) were observed at −30 °C in [$^2H_5$]pyridine and these collapsed to two singlets (δ 1·55 and 1·90) at 80 °C. The ^{1}H n.m.r. signals associated with the aliphatic protons at C-2, C-3, and C-4 on the heterocyclic ring were invariant during these changes, indicating therefore that the shape of this ring remains fixed. These phenomena have thus been interpreted in terms of hindrance to rotation of the aryl group at C-4 about the linkage to C-4. Molecular models show that this is caused primarily by steric interference between the proton at C-2 and the bulky substituents *ortho* to the linkage on the phloroglucinol ring (**22**). Free energies of activation $\Delta G^{\ddagger}_{rot}$ for the rotational barrier were determined[10] and are shown in parentheses for each substrate (kcal mol^{-1}). A complete line shape analysis for (**11**) gave a value of $\Delta S^{\ddagger}_{rot}$ −8·5 cal K^{-1} mol^{-1} and hence it is assumed that a strict comparison of values of the free energy of activation in this series is not valid.[10]

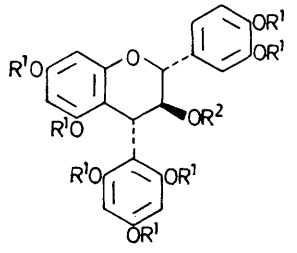
Derivatives (**13**)—(**18**) of the pentahydroxyflavan related to (−)-epicatechin (**5**) were made by reaction of the carbocation (**7**), derived from the polymeric procyanidin in *Butea frondosa*,[11,12] with phloroglucinol or 2,4,6-trihydroxytoluene. These compounds are formulated on the basis of ^{13}C n.m.r. data[12] and earlier arguments[4] as (4*R*)-derivatives and they displayed ^{1}H n.m.r. behaviour entirely analogous to the flavan derivatives (**9**) and (**10**) and consistent there-





	R^1	R^2	$\Delta G^{\ddagger}_{rot}$ /kcal mol^{-1}
(**9**)	H	H	—
(**10**)	H	Et	—
(**11**)	Ac	Ac	14·9[a]
(**12**)	Ac	Et	15·4[b]

	R^1	R^2	R^3	$\Delta G^{\ddagger}_{rot}$ /kcal mol^{-1}
(**13**)	H	H	H	13·9[c]
(**14**)	Ac	Ac	H	19·3[d]
(**15**)	Me	H	H	17·1[e]
(**16**)	H	H	Me	—
(**17**)	Ac	Ac	Me	20·3[d]
(**18**)	Me	H	Me	16·8[e]



	R^1	R^2	$\Delta G^{\ddagger}_{rot}$ /kcal mol^{-1}
(**19**)	H	H	15·7[c] 16·6[f]
(**20**)	Ac	Ac	19·8[d]
(**21**)	Me	H	16·0[a]

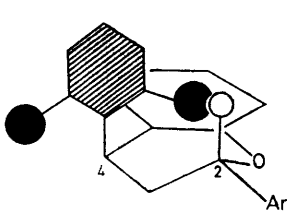
[a] C_5D_5N; [b] $CDCl_3$; [c] $(CD_3)_2CO$; [d] $C_6D_5NO_2$; [e] $CDCl_3$–C_5D_5N; [f] $(CD_3)_2SO$.

fore with hindered rotation about the aryl–C–4 bond. A corollary of these observations is that in the 2,4-*trans*-4-aryl flavans (**9**)—(**18**), over the temperature ranges studied, inversion of the chair conformation of the heterocyclic ring does not take place and the 4-aryl substituent retains a pseudo-axial position.

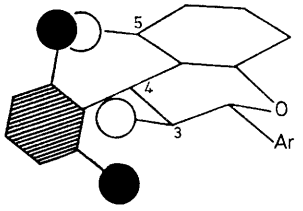
Reaction of the carbocation (**8**), derived from the polymeric procyanidin in *Salix. caprea*,[12] with phloroglucinol gave the 2,4-*cis*-4-arylflavan (**19**) in which the aryl substituent has the (4*S*)-configuration and adopts a pseudo-equatorial position. The phenol (**19**), acetate (**20**), and methyl ether (**21**) all display ^{1}H n.m.r. features characteristic of steric hindrance to rotation about the aryl–C-4 linkage and values for $\Delta G^{\ddagger}_{rot}$ are quoted. The structural situation is analogous to that of the 9-arylfluorene system[13] and models indicate that the oxygen substituents at C-3 and C-5 and in the *ortho* positions to the linkage in the phloroglucinol ring are responsible for the hindered rotation (**23**).

The structures (**9**)—(**18**) provide models from which the conformational properties of the natural procyanidins B-1 and B-2 may be interpolated. Thus for procyanidin B-2 first-order spectra were observed and energies of activation

for rotation about the interflavan bond were estimated as follows: phenol, [$^2H_6$]acetone at 30 °C, 14·9 kcal mol^{-1}: deca-acetate, [$^2H_5$]nitrobenzene at 180 °C, 19·5 kcal mol^{-1}; octamethyl ether, [$^2H_5$]nitrobenzene at 180 °C, 18·7 kcal mol^{-1}. Analogously the phenol (**19**) is a good model for procyanidins B-3 and B-4 and for the former procyanidin first-order spectra and energies of activation for rotation about the interflavan bond were estimated as follows: phenol, [$^2H_6$]dimethyl sulphoxide at 110 °C, 19·1 kcal mol^{-1}; deca-acetate, [$^2H_5$]nitrobenzene at 180 °C, 20·0 kcal mol^{-1}; octamethyl ether, [$^2H_5$]nitrobenzene at 180 °C, 17·9 kcal mol^{-1}. These observations clarify many of the anomalies noted during earlier structural work.[1,2,5–7] They also show that although conformational isomers of the natural procyanidins themselves may be observed spectroscopically these isomers are not separable under normal conditions. However, the acetate and methyl ether derivatives may be capable of the phenomenon of atropisomerism, although no such example has yet been described.[14,15]



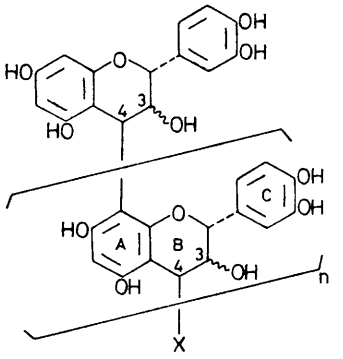
(22)



(23)

Although the structure of the dimeric procyanidins has received most attention to date, analysis of the procyanidin profile in many plants shows that the various polymeric forms (broadly classes *a* and *b* in the Robinsons' classification[16]) are usually quantitatively of greatest metabolic significance to the plant. The available evidence supports the view that they are formed biosynthetically by the multiple self-condensation of the appropriate carbocation[4] following an initial reaction with an appropriate nucleophile (*e.g.* a flavan-3-ol or a saccharide, X). With a single carbocation (**7**) or (**8**) structures of two general types result (**24**) and (**25**).

Elaboration of these polymeric structures, bearing in mind the conformational restraints due to restricted rotation about the interflavan bond outlined above, leads to two similar structures. The central linear core is composed of rings A and B of the flavan repeat unit. The 3′,4′-dihydroxyphenyl units (ring C) project laterally from this thread and their arrangement describes a regular helical conformation; *that of the polymer related to* (+)-*catechin* (**24**) *is a helix with a right-hand screw and that of the polymer derived from* (−)-*epicatechin* (**25**) *is a helix with a left-hand screw.* Whatever the evolutionary and hence biological significance of these helical structural forms (it has been proposed[17] for example that the spatial juxtaposition of the 3′,4′-dihydroxyphenyl groups is most important in the distinctive procyanidin–protein interaction which



(24), 3β-OH, (4*S*)
(25), 3α-OH, (4*R*)

determine astringency and taste) it is nevertheless interesting to note a further example of the economy of Nature. Thus different plants have evolved the means of synthesis of two isomeric polymers possessing opposite helicities by the simple expedient of a change of stereochemistry of the hydroxy-group at C-3 in the carbocation precursor (**7**) and (**8**).

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