

Conformational isomerism of phenolic procyanidins: preferred conformations in organic solvents and water



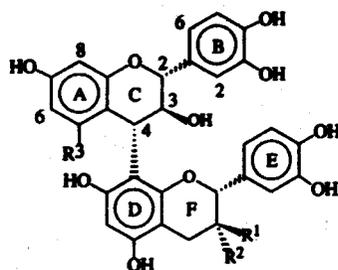
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NMR studies of catechin-(4 α →8)-epicatechin (1) and catechin-(4 α →8)-catechin (2) provided complete assignment of the proton and carbon resonances for both the more extended and compact conformers in the free phenolic form. When 1 is in organic solvents, the more extended rotamer is preferred over the more compact rotamer (10:7), but in water, the compact rotamer dominates (2:10). When 2 is in organic solvents, the more compact rotamer is slightly preferred (8:10), but in water, only trace amounts of the more extended rotamer are detected. NOE experiments show rotational conformation exchange despite the fact that two distinct and sharp sets of signals are seen for each rotamer. The upper unit heterocyclic ring exists in an approximate half-chair conformation in each rotamer for both dimers. However, coupling constants of the lower unit heterocycles show substantial axial orientation of the B-ring. Lineshape analysis of the terminal unit H-3_F excludes E- and A-conformational interchange. These results and NOE experiments show a skewed-boat conformation for the terminal unit in 1 and between a half-chair and skewed-boat conformation for the terminal unit in 2.

Condensed tannins are widely distributed in plants where they are often present as major constituents in tissues surrounding the vascular cambium (tree bark) or membranes surrounding fruits (e.g. peanut or pecan nuts) otherwise exposed to aggressive microbial, insect or animal predation.¹⁻³ The protective properties of condensed tannins are thought to reside in their propensity to complex with proteins. Believing that complexation of polyflavanoids with proteins is, to a significant extent, controlled by conformational preferences and flexibility of the polyflavanoid,⁴ we have engaged in a long-term effort to define the conformational properties of polyflavanoids using a variety of molecular mechanics and molecular orbital computations⁵⁻⁸ in combination with crystal structures,⁹⁻¹² time-resolved fluorescence,¹³⁻¹⁴ as well as NMR methods.^{15,16} The polyflavanoids offer especially interesting challenges because of the large number of potential sources of conformational isomerism including the location and stereochemistry of the interflavanoid bond, rotation about the interflavanoid bond and heterocyclic ring geometry.

NMR spectra of the free phenolic forms of 2,3-*trans*-3,4-*trans* dimeric proanthocyanidins such as catechin-(4 α →8)-



	R ¹	R ²	R ³
1	H	OH	OH
2	OH	H	OH
3	OH	H	H

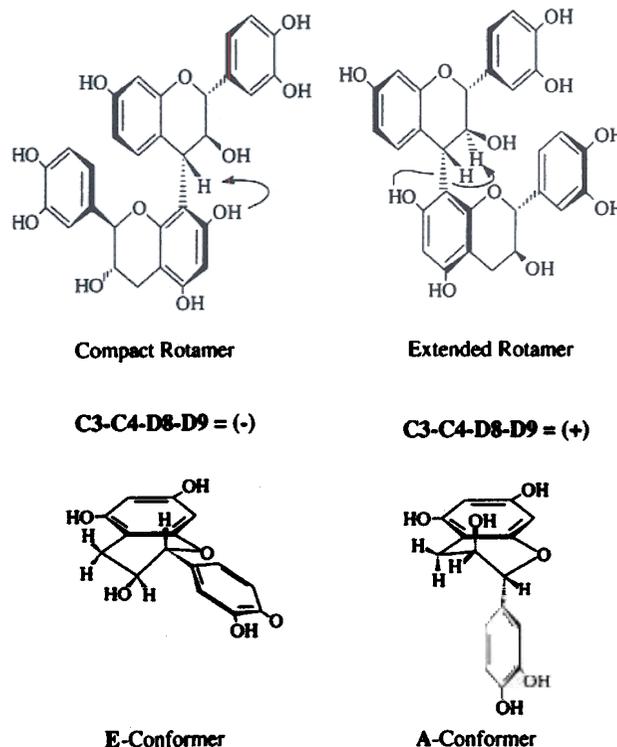


Fig. 1 Examples representing rotational and heterocyclic ring conformational heterogeneity in dimeric procyanidins. Arrows indicate observed NOE in proflisetinidin analogues.⁶

epicatechin (1) or catechin-(4 α →8)-catechin (2) exhibit multiplicity due to rotational isomerism at ambient temperatures (Fig. 1). Fletcher *et al.*¹⁷ studied rotational isomerism in dimeric procyanidins showing that 2 in DMSO exhibited multiplicity in the spectrum observed at 30 °C, but this collapsed to a first-order spectrum at 100 °C. Multiplicity in the NMR spectrum was attributed to rotational isomerism. Foo and Porter¹⁸ expanded our understanding of the conformations of procyan-

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idin dimers in their work on dimers containing either or both 2*R* and 2*S* absolute stereochemistry in the constituent flavan-3-ols. In the phenolic form of the 'normal' dimers (both upper and lower units either 2*R* or 2*S*), the NMR spectra were first-order in compounds with 2,3-*cis* stereochemistry of the upper unit. However, in the 'crossed' dimers (upper and lower units of opposite absolute configuration, *i.e.* 2*R* upper and 2*S* lower units), considerable broadening or multiplicity was evident in ¹³C NMR spectra in compounds with 2,3-*cis* stereochemistry of the upper unit. The increase in restriction of rotation was attributed to closer approach of the E-ring of the terminal unit to the upper unit.

In addition to rotational isomerism, an important potential source of conformational isomerism is associated with the geometry of the heterocyclic rings (Fig. 1). Foo and Porter,¹⁸ studying the peracetate derivatives of procyanidin dimers, suggested that a C-3_C-sofa conformation best fits the observed ³J_{HH} coupling constants for the upper units with 2,3-*cis* relative stereochemistry. A half-chair conformation seemed to fit best for the upper units with 2,3-*trans* relative stereochemistry, but here *J*_{2,3} coupling constants are not so sensitive to conformational change of the C-ring, so both *J*_{2,3} and *J*_{3,4} couplings are needed to define the conformation. The lower units of these dimers approximated a C-2_C-sofa conformation.

The observation of a 'reverse' half-chair or 'axial' B-ring conformation for the pentaacetate of (+)-catechin in the crystal state, whereas the ¹H NMR spectrum indicated a *J*_{2,3} coupling of *ca.* 6.5 Hz, suggested that the heterocyclic ring might be flexing rapidly in the NMR timescale.¹⁰ In an attempt to explain these *J*_{2,3} coupling constants, Porter *et al.*¹⁹ suggested that these coupling constants should be considered as averages of the E- (B-ring equatorial) and A-conformers (B-ring axial) due to rapid heterocyclic ring conformation interchange on an NMR timescale. Porter *et al.*¹⁹ predicted the ratio of A- and E-conformational isomers based on an arithmetical average of the two conformers in comparison with the observed *J*_{2,3} coupling constants. However, such ratios of A- or E-conformers were not consistent with computed differences in conformational energy.⁶

Through use of molecular dynamics¹¹ and molecular search methods,⁹ it is possible to provide support for heterocyclic ring conformational interchange. Predicted coupling constants for tetra-*O*-methyl-(+)-catechin are in reasonable agreement with those observed, particularly when a Boltzmann-average of the ensemble of conformers as predicted by the GMMX† global search methodology is used to predict observed heterocyclic ring coupling constants.^{8,16} Although well tested on monomeric flavan-3-ols, there are no computational or NMR results supporting such heterocyclic ring conformational interchange in dimeric and larger polyflavanoids.

Previous NMR studies directed towards the assignment of the proton and carbon resonances of each rotamer, as is required if we are to use NMR methods to explore the complexation of dimeric flavanoids with polypeptides,²⁰ are limited to only a few contributions.^{6,15-18} Steynberg *et al.*¹⁵ defined proton spectra of each rotamer of the methylated/acetylated derivatives for a series of proflisetinidin dimers. Long-range COSY experiments permitted definition of protons of the upper and lower units in each rotamer. The rotational state of each rotamer could be defined using NOE experiments that showed the presence or absence of correlation between H-4_C and MeO-7_D. It was then possible to define the heterocyclic ring conformations in the upper and lower units of each rotational isomer from ³J_{HH} couplings. The heterocyclic ring in the upper unit was predominantly in an E-conformation (*i.e.* half-chair for the 2*R*,3*S* isomers and in a 'reverse' half-chair for the 2*S*,3*R* isomers). Comparatively small *J*_{2,3} couplings for the 2,3-*trans* ter-

minal F-rings in some of these isomers suggested an axial orientation of the lower unit E-ring. As was suggested in the work by Foo and Porter¹⁸ on peracetate derivatives of procyanidins, the conformations of the methylated/acetylated derivatives of proflisetinidins¹⁵ seem to favour minimizing the surface area of the molecule. Balas and co-workers^{21,22} have studied the peracetate derivatives of the dimers catechin-(4*α*→8)-catechin and catechin-(4*α*→6)-catechin, as well as the trimer catechin-(4*α*→8)-catechin-(4*α*→8)-catechin. This work demonstrated the power of HMQC and HMBC in conjunction with ¹H-¹H COSY and ROESY experiments for determining the location of the interflavanoid bond, as well as the assignments for proton and carbon resonances of the upper, middle and the lower units of the trimer. The conformations of the heterocyclic rings were approximately half-chairs except for the terminal unit in the trimer which adopted a reverse half-chair conformation in which the H-ring was axial thus resulting in broad singlet for H-2.²²

Progress in obtaining NMR data needed for definition of the conformations of oligomeric proanthocyanidins in the free phenolic form, obviously the most important from the point of view of their possible biological significance, is even more limited. Steynberg *et al.*,⁶ studying fisetinidol-(4*α*→8)-catechin (3) in specially-dried [²H₆]acetone, were able to use NOE from the hydroxy proton at position 7_D to H-4_C in one rotamer and to H-3_C of the other rotamer to define their conformations. The more compact rotamer in which the E-ring is back behind the plane of the A- and C-ring was slightly favoured. Approximate half-chair E-conformations were present in both the upper and lower unit heterocyclic rings (Fig. 1).

The phenolic forms of the dimeric procyanidins catechin-(4*α*→8)-epicatechin (1) and catechin-(4*α*→8)-catechin (2) were studied here. In aprotic solvents, two distinct rotational isomers are observed in the NMR spectra of each of the dimeric procyanidins. Although evidence supporting the fact that the two overlapped spectra are due in fact to rotational isomerism has been presented,¹⁷ definition of the conformations of the two rotamers is lacking for the free phenolic forms of these compounds. If E- and A-conformational interchange does occur, it is to be expected that this will be too rapid to be observed as discrete spectra on an NMR timescale. However, if such conformational interchange does occur, it should be possible to find a summation that would account for the lineshape of the heterocyclic ring couplings. Therefore, the work reported here is directed towards (i) complete and unequivocal assignment of the proton and carbon resonances of the two rotational isomers of each of these procyanidins, (ii) definition of the configuration of each rotational isomer, (iii) definition of the conformation of the heterocyclic rings in the upper and lower unit in each rotational isomer and (iv) definition of the influence of differences in solvents on the conformation of these compounds with particular emphasis on their conformations in water.

Results and discussion

NMR assignments in aprotic solvents

Catechin-(4*α*→8)-epicatechin (1). Assignments of the ¹H and selected ¹³C NMR spectra in [²H₆]dioxane, [²H₆]acetone and D₂O are shown in Tables 1 and 2. These assignments were developed primarily using long-range coupling enhanced ¹H-¹H shift correlation (COSYLR) and heteronuclear ¹H-¹³C correlation (HETCOR) as well as multiple bond connectivity (either COLOC or HMBC) experiments. The protocol used for assignment in the different solvents was similar, so only selected examples will be described in detail.

Because of substantial symmetry and at times overlapping signals, incorrect assignments of the proton and carbon resonances of the 5,7-dihydroxyflavan A-ring in particular are frequently found in published literature.¹⁶ A COLOC experiment

† Mention of trade names does not constitute endorsement by the US Department of Agriculture.

Table 1 ^1H NMR assignments for catechin-(4 α →8)-epicatechin (1)

Solvent	Rotamer	Unit	A-/D-ring		B-/E-ring			C-/F-ring		
			6	8	5	6	3	4		
Dioxane	A [1.00] ^b	U	5.67 (2.3) ^a	5.77 (2.3)	6.89 (2.0)	6.71 (8.0)	6.82 (2.0, 8.0)	4.44 (9.6)	4.34 (m)	4.58 (7.7)
		L	5.74 (s)		7.09 (2.0)	6.72 (8.0)	6.82 (2.0, 8.0)	4.88 (br s)	4.15 (br m)	2.84 (4.5, 16.5)
	B [0.70]	U	5.76 (2.3)	5.81 (2.3)	6.63 (2.0)	6.59 (8.0)	6.46 (2.0, 8.0)	4.33 (9.4)	4.11 (m)	4.33 (8.3)
		L	5.90 (s)		6.54 (2.0)	6.62 (8.0)	6.30 (2.0, 8.0)	4.73 (br s)	3.97 (br m)	2.75 (4.5, 16.5)
Acetone ^c	A [1.00]	U	5.84 (2.4)	5.85 (2.4)	7.01 (2.0)	6.80 (8.0)	6.87 (2.0, 8.0)	4.46 (9.7)	4.55 (9.7, 7.6)	4.70 (7.6)
		L	6.04 (s)		7.21 (2.0)	6.79 (8.0)	6.94 (2.0, 8.0)	5.00 (br s)	4.25 (br m)	2.95 (4.5, 17)
	B [0.75]	U	5.94 (2.4)	5.95 (2.4)	6.86 (2.0)	6.67 (8.0)	6.56 (2.0, 8.0)	4.40 (9.5)	4.36 (m)	4.53 (7.9)
		L	6.20 (s)		6.71 (2.0)	6.71 (8.0)	6.38 (2.0, 8.0)	4.89 (br s)	4.11 (m)	2.85 (4.3, 17)
D ₂ O ^d	A [1.00]	U	5.88 (2.5)	5.80 (2.5)	6.54 (1.9)	6.61 (8.2)	6.32 (1.9, 8.2)	4.29 (9.8)	4.12 (9.8, 8.3)	4.25 (8.3)
	L	6.01 (s)		6.52 (1.9)	6.67 (8.2)	6.28 (1.9, 8.2)	4.68 (br s)	3.97 (br m)	2.74 (4.7, 17.3)	
									2.52 (br d, 17.3)	

^a Data in parentheses are J values in Hz or multiplicity. ^b Data in brackets are relative proportions of the two rotameric forms where A and B conformers are the extended and compact conformers, respectively. ^c Data in dry acetone where OH coupling to H-3_F was evident. Small amounts of D₂O were added to some samples discussed in the text so small deviations in chemical shift and coupling constants will be seen between the data in this table and referenced in the text. ^d Signals for the minor rotamer were too small to permit accurate assignments.

Table 2 ^{13}C NMR assignments for catechin-(4 α →8)-epicatechin (1)^a

Solvent	Rotamer	Unit	A-/D-ring		B-/E-ring		C-/F-ring			
			6	8	5	6	3			
Dioxane	A [1.00] ^b	U	97.0	95.8	115.7	115.5	120.6	83.0	73.5	37.8
		L	97.0		115.0	115.5	119.0	79.7	66.4	29.2
	B [0.70]	U	97.0	96.7	115.7	115.5	120.4	83.1	73.3	38.3
Acetone ^c	A [1.00]	U	97.3	96.2	116.0	115.6	120.8	83.5	73.2	38.3
		L	97.6		115.2	115.5	119.2	80.0	66.9	29.7
	B [0.70]	U	97.4	96.9	116.1	115.6	120.5	83.7	73.2	38.5
D ₂ O ^d	A [1.00]	U	96.5		114.3	115.6	119.7	79.2	67.0	29.1
	L	96.6	95.7	115.5	115.9	119.9	81.6	72.0	36.6	
		L	95.4		114.3	115.1	119.2	77.7	65.6	27.3

^a Data for quaternary carbons are shown in Fig. 2. ^b Data in brackets indicate relative proportions of two conformers in which the A and B conformers are the extended and compact conformers, respectively. ^c Data measured in dry acetone. Small amounts of D₂O were added to some samples discussed in the text so small deviations in chemical shift and coupling constants will be seen between the data in this table and referenced in the text. ^d Signals for the minor rotamer were too small to permit accurate assignments.

on 1 in [$^2\text{H}_6$]acetone optimized for 3 Hz long-range H-C correlation showed that the *meta*-coupled doublet at δ_{H} 5.85 was correlated with the downfield aromatic carbon at δ_{C} 158.4, whereas the Ar-H doublet at δ_{H} 5.84 was correlated with carbon resonances at δ_{C} 157.4 and 157.2, suggesting that the downfield Ar-H must be assigned to H-8_A. However, a crosspeak between H-2_C across the pyran oxygen to C-9_A was not detected. An HMBC experiment (optimized for 5 Hz long-range C-H coupling) on 1 in [$^2\text{H}_6$]acetone showed that the H-2_C doublet at δ_{H} 4.46 ($^3J_{\text{HH}} = 9.7$ Hz) of the major rotamer gave a weak, but clear crosspeak to the carbon at δ_{C} 158.4 (Fig. 2). In addition, the *meta*-coupled doublet at δ_{H} 5.85 was correlated with carbon resonances at δ_{C} 158.4 and 157.4, whereas the doublet at δ_{H} 5.84 showed crosspeaks to carbon resonances at δ_{C} 157.2 and 157.4. Therefore, the H-6_A is assigned the resonance at δ_{H} 5.84 and H-8_A is downfield at δ_{H} 5.85. A HETCOR experiment then established connectivity of H-8_A with the carbon resonance at δ_{C} 96.2 and the H-6_A with the carbon δ_{C} 97.3 (Table 2).

A similar correlation showed that in the minor rotamer in which the H-6_A and H-8_A protons are just barely resolved at δ_{H} 5.94 and 5.95. The corresponding carbon resonances are at δ_{C} 97.4 and 96.9. These results were consistent with a series of selective decoupling experiments that were made to assist in the assignment of the C-5_A, C-7_A and C-9_A carbon resonances. In addition, the relative chemical shifts of the H-6_A and H-8_A protons and corresponding carbon resonances are similar to those

found in spectra measured in [$^2\text{H}_6$]dioxane (Tables 1 and 2). However, these proton assignments are inverse to those found in spectra measured in D₂O.

In an effort to clarify this question, a sample of 1 was added to D₂O and kept at ambient temperature for two days after which it was freeze-dried and the spectrum recorded in [$^2\text{H}_6$]acetone to which various amounts of D₂O were added. When acetone was the solvent, the H-8_A signal was significantly smaller than H-6_A with the chemical shifts similar to those described above. After addition of 11% of D₂O, the H-6_A and H-8_A protons of the minor rotamer had collapsed to a singlet and those of the major rotamer were almost superimposed. Further addition of D₂O to a 20% level collapsed the H-6_A and H-8_A of the major rotamer and two signals began to emerge again for the minor rotamer. Finally, after addition of 77% of D₂O, both the major and minor rotamers once more showed two sets of *meta*-coupled doublets, but the relative order of the chemical shifts of the larger and smaller signals were inverted from those seen when recorded in [$^2\text{H}_6$]acetone. The spectral properties of these compounds in D₂O are discussed more fully below.

The HMBC experiment provides an efficient route to solving the question of the location of the interflavanoid bond,²¹ which has previously been a substantial challenge, often requiring methylation and a series of methoxyl NOE experiments.¹⁵ An HMBC experiment on 1 in [$^2\text{H}_6$]acetone showed a cross peak resulting from the H-2_F which appeared as a broad singlet at δ_{H} 5.00 to C-9_D at δ_{C} 155.0 as was seen in the upper unit of this

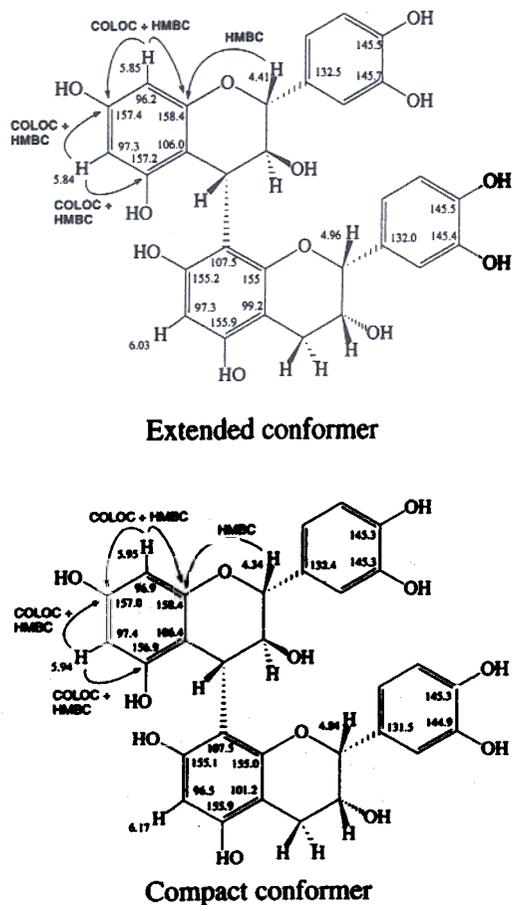


Fig. 2 HMBC correlations used to assign H-6_A and H-8_A in 1

dimer. In addition, the H-4_C doublet at δ_{H} 4.70 ($^1J_{\text{HH}} = 7.6$ Hz) also showed a cross peak to this same carbon. If this compound had been formed with a C-4_C to C-6_D linkage, the Ar-H singlet would have to be assigned to H-8_D and H-4_C could not have correlation with C-9_D.

The HMBC experiment also permitted assignment of the C-1_B and C-1_E signals through correlations with H-2_C and H-2_F protons. These correlations showed that the higher field carbons at δ_{C} 131.5 and 132.0 were the C-1_E carbons of the lower unit of the minor and major rotamers, respectively. The C-1_B carbons of the upper unit of minor and major rotamers appeared at δ_{C} 132.4 and 132.5, respectively. The resolution in the HMBC experiment was not sufficient to assign the C-3 and C-4 carbons of the B- and E-rings. However, a COLOC experiment optimized at 8 Hz coupling showed correlations between the H-2_E and H-2_B protons of the more extended major rotamer at δ_{H} 7.21 and 7.01 with the carbon resonances at δ_{C} 145.4 and 145.7 for C-3_E and C-3_B, respectively. The slightly resolved H-5 doublets ($J = 8$ Hz) of this rotamer for the upper and lower units at δ_{H} 6.80 and 6.79 showed strong cross peaks with carbons at δ_{C} 145.52 and 145.48 for C-4_B and C-4_E, respectively.

Because dioxane is the preferred solvent for fluorescence studies, NMR spectra were assigned in [²H₆]dioxane as well. The relative proportions of the two rotamers as well as chemical shifts and coupling constants for each of the two rotamers were similar when recorded in either [²H₆]acetone or [²H₆]dioxane (Table 1). However, interpretation of the proton coupling patterns seen for 1 in [²H₆]dioxane was more difficult. For example, the presence of two hydroxy proton signals overlapping the B- and E-ring protons complicated direct assignment, but the couplings were readily sorted out using a *J*-resolved experiment. Hydroxy coupling to H-3_C in both rotamers resulted in very broad complex multiplets, but their assignment could be made using a series of HOMODEC experiments in

which each of the H-2_C, H-3_C and H-4_C protons were irradiated. When the spectrum of a sample of 1 which had been partially exchanged with deuterium was compared with a normal sample in [²H₆]dioxane, the H-8_A signals were clearly depressed more than the H-6_A signals.

Catechin-(4 α →8)-catechin (2). A similar protocol was used to assign the proton and carbon resonances of 2 summarized in Tables 3 and 4. As before, an HMBC experiment was used to assign the H-6_A and H-8_A of the two rotamers. An interesting problem in interpreting the C-ring coupling patterns arose here because small amounts of D₂O were added to the acetone to eliminate hydroxy signals and coupling to H-3_C. This caused overlap of H-3_C and H-4_C in both rotamers and that resulted in 'virtual coupling' of the H-2_C of both rotamers. The H-2_C signal was converted to the expected doublet by adding less or more D₂O to the solvent. Having resolved that question, it was then possible to assign the A-ring Ar-H protons for the major rotamer using HMBC experiments. The cross peaks from H-2_C at δ_{H} 4.28 as well as H-8_A at 5.83 to C-9_A at δ_{C} 158.6 clearly established the assignment of H-8_A for the major rotamer. This spectrum also showed a weak cross peak between H-2_C of the minor rotamer at δ_{H} 4.39 and the C-9_A of this rotamer at δ_{C} 158.5; but the H-6_A and H-8_A protons for the minor rotamer were nearly superimposed, so it was not possible to distinguish between the two because of insufficient resolution in the proton dimension.

To solve this problem, a sample of 2 was added to D₂O and allowed to stand at room temperature for 2 days to deuteriate partially the A- and D-ring protons. In contrast to observations on the relative rates of exchange of H-6_A and H-8_A observed in (+)-catechin and 1, the H-6_A proton in the major rotamer 2 (the assignment clearly defined by a HMBC experiment) was exchanged more than H-8_A. The proton spectrum of the partially deuterium-exchanged sample then appeared at two comparatively sharp singlets for the H-8_A protons at δ_{H} 5.86 and 5.83 with smaller and more broadened signals for the H-6_A protons at δ_{H} 5.93 and 5.85 for the major and minor rotamers, respectively.

The HMBC experiment also verified that both rotamers were linked through 4→8 interflavanoid bonds because of cross peaks between H-4_C of both the major and minor rotamers at δ_{H} 4.46 and 4.57, with the C-9_D carbons at δ_{C} 154.9 and 154.7 that were defined by correlation to the H-2_F protons at δ_{H} 4.57 and 4.73, respectively. Long-range COSY experiments working from H-4_C and the two H-4_F protons distinguished the H-2_C and H-2_F protons of each rotamer, and correlation of those with H-2_B and H-6_B or H-2_E and H-6_E permitted assignment of the two catechol rings to the upper and lower unit in each rotamer. For example, the H-2_C of the major rotamer showed crosspeaks with the H-2_B doublet at δ_{H} 6.78 and to the H-6_B double doublet ($J = 2.0$ and 8.0 Hz) at δ_{H} 6.50, whereas the H-2_F doublet of the lower unit of this rotamer showed crosspeaks with the H-2_E doublet at δ_{H} 6.65 and to the H-6_E double doublet at δ_{H} 6.29. The other rotamer H-2_C proton showed a crosspeak with the H-2_B doublet at δ_{H} 6.99 and was correlated with the H-2_F at δ_{H} 4.73 lowest field H-2_E doublet at δ_{H} 7.03 and to the H-6_E double doublet at δ_{H} 6.88. The H-5_B and H-5_E doublets were then assigned from a long-range COSY experiment. These assignments were supported by a series of selective homonuclear decoupling experiments as well as by *J*-resolved experiments. The corresponding carbon assignments were then made using HETCOR and HMBC experiments.

The spectra of 2 in [²H₆]dioxane also highlight the difficulty in accurately assigning the H-6_A and H-8_A of the two rotamers in the phenolic form. Three of the A-ring protons are overlapped at *ca.* δ_{H} 5.74, so a *J*-resolved experiment was used to show that the H-6_B protons of the major and minor rotamers were at δ_{H} 5.87 and 5.73 and that two of the *meta*-coupled doublets were superimposed at δ_{H} 5.75. The other two *meta*-coupled doublets, one belonging to the major rotamer and the

Table 3 ¹H NMR assignments for catechin-(4 α →8)-catechin (2)

Solvent	Rotamer	Unit	A-/D-ring		B-/E-ring			C-/F-ring		
			8	5	6	3	4			
Dioxane	A [1.00] ^b	U	5.75 (2.4) ^a	5.69 (2.4)	6.62 (2.0)	6.60 (8.0)	6.41 (2.0, 8.0)	4.23 (9.5)	4.13 (m) ^a	4.27 (7.5)
		L	5.87 (s)		6.50 (2.0)	6.63 (8.0)	6.23 (2.0, 8.0)	4.43 (7.5)	3.69 (m)	2.77 (5.5, 16)
	B [0.70]	U	5.66 (2.4)	5.75 (2.4)	6.88 (2.0)	6.71 (8.0)	6.80 (2.0, 8.0)	4.38 (9.5)	4.33 (m) ^a	4.46 (7.5)
		L	5.73 (s)		6.89 (2.0)	6.70 (8.0)	6.81 (2.0, 8.0)	4.67 (7.5)	3.96 (m)	2.77 (5.5, 16)
Acetone ^c	A [1.00]	U	5.94 (2.4)	5.83 (2.4)	6.78 (2.0)	6.68 (8.0)	6.50 (2.0, 8.0)	4.28 (9.0)	4.40 (7.5, 9.0)	4.46 (7.5)
		L	6.18 (s)		6.65 (2.0)	6.69 (8.0)	6.29 (2.0, 8.0)	4.57 (7.5)	3.80 (m)	2.80 (5.5, 16)
	B [0.80]	U	5.85 (2.4)	5.86 (2.4)	6.99 (2.0)	6.80 (8.0)	6.84 (2.0, 8.0)	4.39 (9.5)	4.56 (m)	4.57 (m)
		L	6.05 (s)	—	7.03 (2.0)	6.77 (8.0)	6.88 (2.0, 8.0)	4.73 (7.0)	4.06 (m)	2.90 (5.5, 16)
D ₂ O ^d	A [1.00]	U	5.83 (2.4)	5.54 (2.4)	6.63 (2.0)	6.69 (8.0)	6.44 (2.8)	4.21 (9.8)	4.10 (7.7, 9.8)	4.19 (7.7)
	L	5.98 (s)		6.44 (2.0)	6.66 (8.0)	6.29 (2.8)	4.39 (8.1)	3.73 (m)	2.67 (5.6, 16)	
									2.31 (9.0, 16)	

^a Data in parentheses are *J* values in Hz or multiplicity. ^b Data in brackets are relative proportions of the two rotameric forms in which the A and B conformers are the compact and extended conformers, respectively. ^c Data measured in dry acetone where OH coupling to H-3_F was evident. Small amounts of D₂O were added to some samples discussed in the text so small deviations in chemical shift and coupling constants will be seen between the data in this table and referenced in the text. ^d Signals for the minor rotamer were too small to permit accurate assignments.

Table 4 ¹³C NMR assignments for catechin-(4 α →8)-catechin (2)^a

Solvent	Rotamer	Unit	A-/D-ring		B-/E-ring		C-/F-ring			
			6	8	6	3	4			
Dioxane	A [1.00] ^b	U	96.7	96.5	115.8	115.5	120.4	83.1	73.5	37.8
		L	95.8		115.2	115.5	119.7	82.3	68.1	29.2
	B [0.70]	U	97.0	97.0	115.7	115.5	120.6	83.2	73.2	38.3
		L	95.8		115.3	115.3	119.9	82.6	67.9	28.9
Acetone ^c	A [1.00]	U	97.0	96.8	116.1	115.6	120.5	83.7	73.3	38.2
		L	96.2		115.3	115.7	119.7	82.3	68.4	28.9
	B [0.75]	U	97.3	96.2	115.9	115.6	120.8	83.8	73.1	38.1
		L	97.5		115.0	115.8	120.1	83.1	68.2	28.8
D ₂ O ^d	A [1.00]	U	96.2	95.	115.4	115.8	120.0	81.4	72.2	36.5
	L	95.0		114.6	115.3	119.6	80.0	66.6	26.8	

^a Data for quaternary carbons are shown in Fig. 2. ^b Data in brackets indicate the relative proportions of two rotamers in which the A and B conformers are the compact and extended conformers, respectively. ^c Data measured in dry acetone. Small amounts of D₂O were added to some samples discussed in the text so small deviations in chemical shift and coupling constants will be seen between the data in this table and referenced in the text. ^d Signals for the minor rotamer were too small to permit accurate assignments.

other to the minor rotamer on the basis of their integration, were at δ_{H} 5.66 and 5.69. The partially deuterium-exchanged sample of 2 described above was also used as a probe for assignment of the A-ring protons in [²H₆]dioxane. Here, the two H-8_A singlets (see discussion of spectral assignments in acetone above) remained as strong signals, one at δ_{H} 5.75 and the other at δ_{H} 5.69. A HETCOR experiment showed that the H-8_A of the major rotamer at δ_{H} 5.69 was correlated with a carbon resonance at δ_{C} 96.5 and the H-8_A of the minor rotamer at δ_{H} 5.75 was correlated with a carbon resonance at δ_{C} 97.0. The two H-6_A protons at δ_{H} 5.75 and 5.66 were correlated with carbon resonances at δ_{C} 96.7 and 97.0 for the major and minor rotamers, respectively.

To explore these assignments further, 2 was added to [²H₆]dioxane and proton spectra were recorded after incremental dropwise addition of D₂O where the A- and D-ring proton signals shifted downfield. With the addition of 6 drops of D₂O, the chemical shifts of H-6_A and H-8_A of the major rotamer now appeared at δ_{H} 5.83 and 5.73. The corresponding chemical shifts of the minor rotamer were δ_{H} 5.74 and 5.79, similar to the relative order observed in dry [²H₆]dioxane. When recorded in D₂O, one rotamer was observed and here the relative order of the chemical shifts of H-6_A and H-8_A were inverted at 5.83 and 5.54, respectively. Assignment of the B- and E-ring protons required a series of HOMODEC experiments, but once the proton signals were assigned the protonated carbon resonances were readily assigned with a HETCOR experiment.

Rotamer conformations

An important feature of the rotational isomerism in the free phenols 1 and 2 was the 'rotational conformation exchange' first noted in NOESY experiments of 1 when recorded in [²H₆]acetone with a small amount of D₂O. These spectra showed that strong crosspeaks representing conformational exchange in which correlations between the same proton in each of the two rotameric isomers dominate the spectrum. This effect was explored further in NOE-difference experiments, which clearly showed that despite the sharp signals for each of the two rotamers, the effect of irradiation of a proton in one rotamer was translated to the same proton in the other rotamer, indicating that there is rotational interchange between two conformers in the timescale of this experiment.

Long-range COSY experiments showed strong crosspeaks due to five-bond coupling between H-4_C and the A-ring Ar-H protons of each rotamer pair. This is analogous to allylic coupling where the correlation is maximized when the dihedral angles between the C-H σ -bond and the π orbitals are 0°. The H-6_A and H-8_A (the A-ring plane), form an approximate 90° orientation relative to H-4_C (approximately axial to the heterocyclic C-ring plane). Hence, both rotamers show strong crosspeaks between H-4_C and H-6_A or H-8_A. The power of this experiment lies in its sensitivity to the orientation of H-4_C with the plane of the D-ring as evidenced through correlations with H-6_D. For 1 in [²H₆]acetone, strong crosspeaks between H-4_C and both H-6_A and H-8_A as well as H-6_D in the major rotamer indicate that it was the rotamer in which the angle between

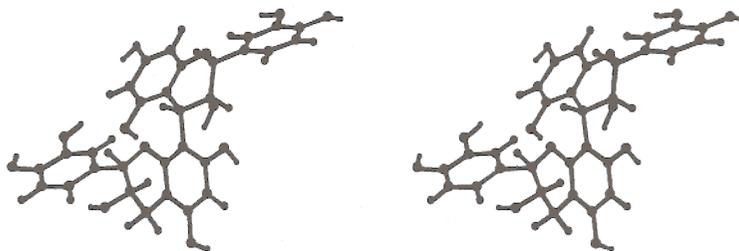


Fig. 3 Stereoview of the extended rotamer of 1

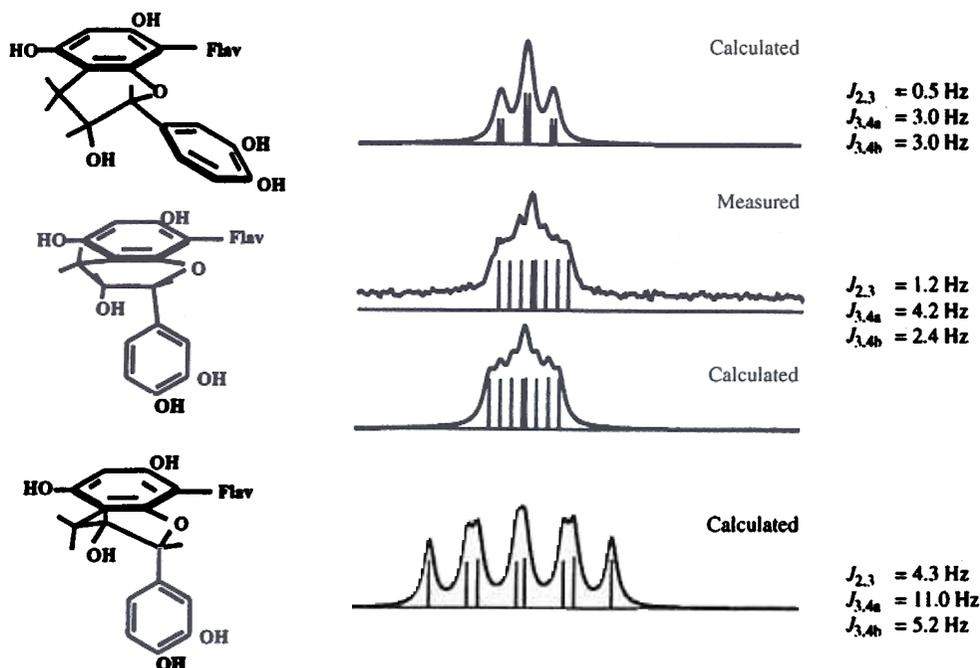


Fig. 4 PCPMR lineshape analyses of the H-3_F proton in 1 when recorded in [2H₆]acetone with a small amount of D₂O

H-4_C and H-6_D was *ca.* 90°. The minor rotamer showed only a weak crosspeak between these two protons, suggesting an angle between H-4_C and H-6_D deviating towards either 0° or 180°. Although the relative populations of the two rotamers were similar, the situation was different for 2 in [2H₆]acetone. Here, the major rotamer showed strong crosspeaks between H-4_C and both the H-6_A and H-8_A protons, but only a very weak crosspeak could be seen between H-4_C and H-6_D. By contrast, the H-4_C of the minor rotamer showed strong crosspeaks to H-6_A and H-8_A as well as an approximately equally strong crosspeak to H-6_D, suggesting an approximate 90° orientation of the H-6_D to H-4_C.

The orientation of the two rotational conformers was further investigated using NOESY and NOE-difference experiments. In addition to the strong crosspeaks attributable to conformational exchange, the expected correlations between H-2_C and H-2_F with the corresponding B- and E-ring H-2 and H-6 protons help to assign the upper and lower unit catechol rings. The most important observation is the NOE correlation seen between H-2_E and H-4_C in the major rotamer of 1 and in the minor rotamer of 2. These correlations were studied further in NOE-difference spectra. Irradiation of the major H-2_E of 1 resulted in a strong positive NOE with H-4_C as well as a negative (conformational exchange) signal for H-2_E of the minor rotamer.

By contrast, irradiation of the H-2_E of the minor rotamer of 2 resulted in comparatively weak positive NOE with both H-2_F and H-4_C of the minor rotamer and a stronger negative (conformational exchange) signal for H-2_E of the major rotamer. Differences between 1 and 2 are consistent with relaxation of the irradiation in 1 being directed more through the correlation between H-2_E and H-4_C than in 2 where more of the saturation was transferred to the other rotamer

through conformational exchange. The relative intensities of the crosspeaks between H-4_C and H-6_D for the two rotamers seen in the long-range COSY experiment, the NOE seen between H-2_E and H-4_C, and the coupling constants found for the terminal unit heterocyclic rings (discussed more fully below) in both 1 and 2 are all consistent with a conformation such as shown in Fig. 3 for the more extended rotamer in [2H₆]acetone.

When examined using the MM2 molecular mechanics force-field, two rotational isomers with only small (*ca.* 3.7 kcal mol⁻¹) differences in conformational energy are evident. For the 4→8 linked dimers, the C3-C4-D8-D9 torsion angles centre on ±90°. However, the flexibility in rotation is limited to a narrow range of from ±75 to ±104° for acceptable energy minima because of steric interactions.³ The high energy barrier to rotation through 360° coupled with the observation of conformational exchange between the two rotamers seen in NOE experiments begs the question of whether the two 'rotamers' correspond to conformations in which the C3-C4-D8-D9 torsion angles centre on ±90° or if these two conformations are represented by two stabilized forms, the 'extended' conformer in which the C3-C4-D8-D9 torsion angle is *ca.* -180° and the more 'compact' rotamer in which the π-π interaction²³ between the B- and E-rings would stabilize the conformation where the C3-C4-D8-D9 torsion angle is *ca.* +10°. Both of these conformations are consistent with the relative intensities of the H-4_C and H-6_D crosspeaks observed in long-range COSY experiments. The conformation of the more extended rotamer is supported by the observation of NOE between the H-2_E and H-4_C protons (Fig. 3).

The conformation of the more compact rotamer is supported by the anisotropy demonstrated by the large difference in chemical shifts of the B- and E-rings of the two rotamers, particularly for H-2_E and H-6_E. Note that the E-ring is positioned in a plane close to that of the B-ring in the compact conformers and that it is not necessary to rotate 360° through the large energy barrier involved in aligning the A- and D-rings to interchange between the extended and compact conformers. Because the conformation of the heterocyclic ring in the terminal unit is so important to the stabilization of these two rotational conformations, considerable efforts were made to evaluate the F-ring conformations.

Heterocyclic ring conformations

In either [²H₆]acetone with a small amount of D₂O added to suppress hydroxy signals or in [²H₆]dioxane, the coupling constants observed for the upper unit heterocyclic ring in the extended rotamer of 1 were in reasonably close agreement with a half-chair E-conformation, but with a slight distortion

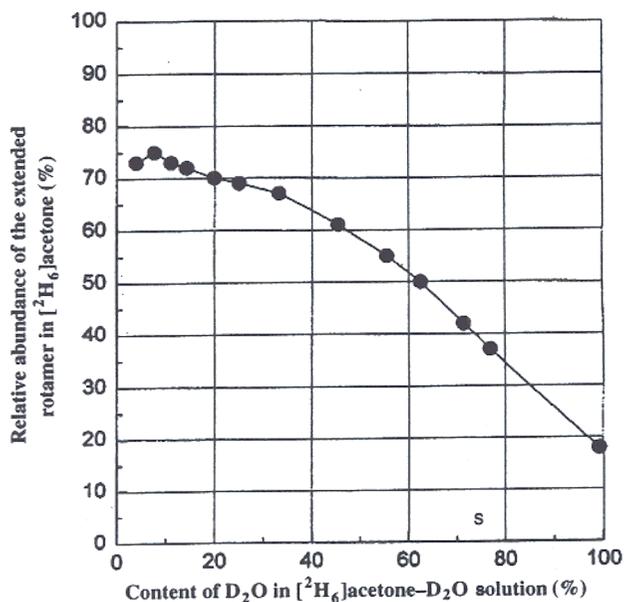


Fig. 5 Effect of D₂O concentration on the relative proportions of the extended and compact rotamer of 1

toward a C-3_C-sofa resulting from rocking of the lower unit slightly back behind the plane of the A- and C-rings, hence a smaller $J_{3,4}$ coupling constant than would be predicted of a true half-chair (Table 1). The coupling constants suggest that the heterocyclic C-ring in the minor more compact rotamer was distorted slightly more to a C-2_C-sofa. The upper unit of both rotamers of 2 had coupling constants that indicated a conformation close to a half-chair with a slight distortion to a C-2_C-sofa (Table 3).

However, the coupling constants observed for the terminal units in both rotamers of either 1 or 2 were substantially different from those expected of half-chair conformations. Given the previous evidence for heterocyclic ring conformational interchange, we first assumed that these coupling constants might be explained on the basis of E- and A-conformational averaging in the NMR timescale. Lineshape analysis of the observed H-3_F multiplet using PCPMR,²⁴ in conjunction with the coupling constants predicted for the E- and A-conformers of the terminal flavan-3-ol when minimized using the MMX force field in PCModel,²⁵ showed that the observed H-3_F coupling could not be accounted for by any summation of the E- and A-conformers as would be required if the heterocycle flipped back and forth between these two low energy states (Fig. 4).

Instead, the observed coupling constants suggested a comparatively high energy (5–7 kcal mol⁻¹) skewed-boat conformation for the lower unit heterocycle. Analysis of the H-3_F of the major (more extended) rotamer of 1 in [²H₆]acetone plus a small amount of D₂O is offered as an example. When optimized using the MMX force field in PCModel, the E-conformer of epicatechin would have a $J_{2,3}$ value of 0.5 Hz and both $J_{3,4a}$ and $J_{3,4b}$ would be ca. 3.0 Hz. In the A-conformation in which the B-ring is axial, $J_{2,3}$ would be expected to be 4.3 Hz with $J_{3,4a}$ at 11.0 Hz and $J_{3,4b}$ at 5.2 Hz. When compared with the observed H-3_F lineshape, it is clear that the comparatively large $J_{2,3}$ observed (1.2 vs. 0.5 Hz) cannot be attributed to the presence of the A-conformer because that would result in a much broader splitting pattern than was observed. The observed H-3_F lineshape can be closely matched using PCPMR particularly with the aid of known $J_{3,4a}$ and $J_{3,4b}$ coupling constants that were readily obtained from the spectrum. This provided an estimate of $J_{2,3}$ of 1.2 Hz which, when combined with the $J_{3,4}$ couplings, indicated a skewed-boat conformation for the heterocyclic F-ring. The analyses of Steynberg *et al.*⁶ of the energy barriers to E- and A-conformational interchange suggest that the

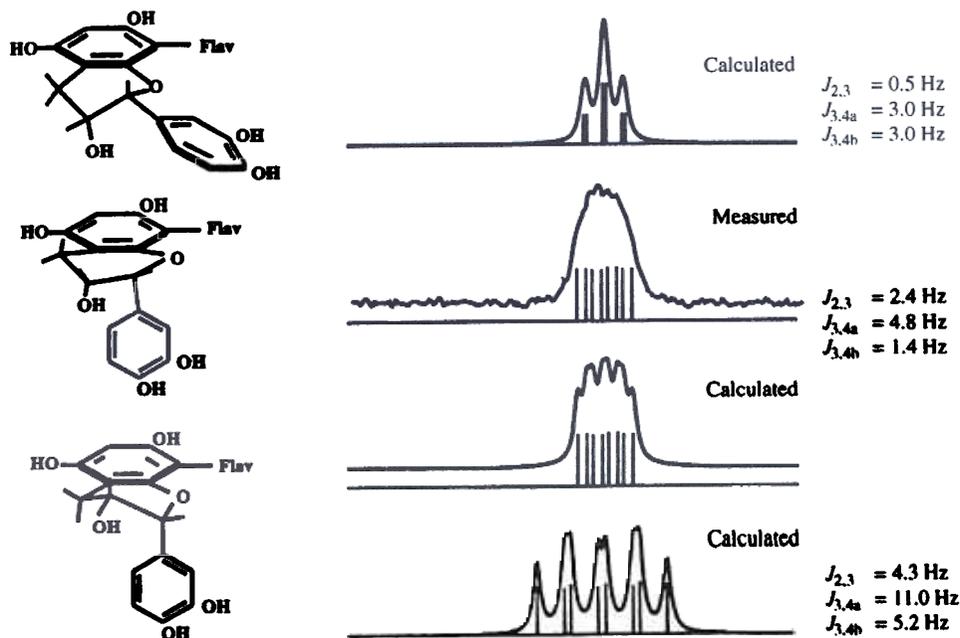


Fig. 6 PCPMR lineshape analyses of the H-3_F proton in 1 when recorded in D₂O

energy of this skewed-boat conformation might be *ca.* 6 kcal mol⁻¹ higher than for the lowest energy half-chair conformation.

Note that the prediction of a skewed-boat conformation of the terminal epicatechin unit in **1** from the F-ring coupling constants is also supported by NOESY and NOE-difference experiments that showed correlation between H-2_E and H-4_C for this same rotamer. The latter observation requires that the E-ring is in an axial position in order to align closely these two protons. Additional support for this F-ring conformation comes from a long-range COSY experiment that showed a strong crosspeak between H-4_C and H-6_D, suggesting an approximate 90° orientation of these two protons. Consideration of the observed coupling constants for the minor and more compact rotamer of **1** also shows a skewed-boat conformation in this rotamer which when combined with the observation of distortion to a C-2_C-sofa in the upper unit provides appropriate orientation for π-π interaction of the B- and E-rings when the torsion angle H4-C4-D8-D7 is *ca.* +60°. This would account for the strong up-field shift of the proton resonances of the B- and E-rings in this rotamer.

The conformation of the terminal unit in **2** differs somewhat from that in **1** with apparently more half-chair character as evidenced by lineshape analysis of H-3_F. However, NOESY and NOE-difference spectra show correlations between H-2_E and H-4_C that can best be accounted for by a skewed boat conformation of the F-ring. PC Model shows that the hydroxy group at 3_C has a tendency to form hydrogen bonding with the pyran oxygen of the F-ring and this places the E-ring slightly behind the B-ring.

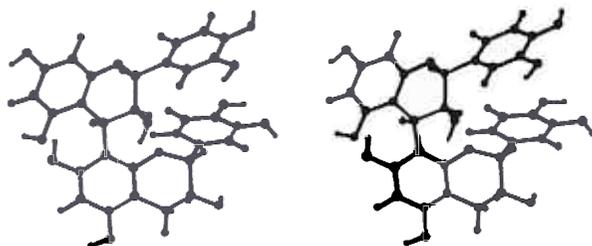


Fig. 7 Stereoview of the compact rotamer of **1**

Conformations in water

Obviously, from the standpoint of helping to understand the biological properties of polyphenols, the important conformations are those of the free phenols in water. As was observed by Haslam and co-workers¹⁷ for **2** and Steynberg *et al.*⁶ for **3**, the proton spectra of both **1** and **2** showed predominantly one rotamer when the spectra are recorded in D₂O. Studies of the relative proportions of the more extended rotamer with normal catechol B- and E-ring chemical shifts, and the more compact rotamer in which the B- and E-ring protons are shifted upfield of **1** in [²H₆]acetone containing different portions of D₂O show that in [²H₆]acetone the more extended rotamer is present in *ca.* 65% and the more compact rotamer in *ca.* 35% of the population. Addition of small amounts of D₂O (up to *ca.* 10%) resulted in a slight increase in the population of the more extended rotamer. However, further addition of D₂O resulted in a gradual decline in the proportion of the more extended rotamer with corresponding increase in the signals due to the more compact rotamer (Fig. 5). When **1** is in D₂O, the more extended rotamer occupies less than 20% of the population. Similarly, when **2** is in [²H₆]acetone, the more extended rotamer is the minor rotamer being present in only *ca.* 40% of the population. However, when measured in D₂O, only signals representing the more compact rotamer are seen. Using the MMX force-field in PCModel, the more extended rotamer appears to be stabilized by hydrogen bonding of the aliphatic hydroxy group at C-3_C with the aromatic hydroxy group at C-7_D. The more compact rotamer shows hydrogen bonding between the aliphatic hydroxy group at C-3_C and the pyran oxygen of the F-ring. When the sample is dissolved in water, the hydrogen bonding restraints should be eliminated allowing the more compact rotamer to dominate, apparently in a comparatively high energy skewed-boat conformation because of π-π interaction between the B- and E-rings.

Lineshape analysis of the H-3_F protons of **1** in D₂O again support the thesis that the F-ring coupling constants cannot be interpreted as conformational interchange between E- and A-conformers, but rather that comparatively high energy skewed-boat conformations must dominate (Fig. 6). For the more compact rotamer that is predominant when either of these compounds is dissolved in water, such a high energy state might be stabilized by π-π interaction of the B- and E-rings. However,

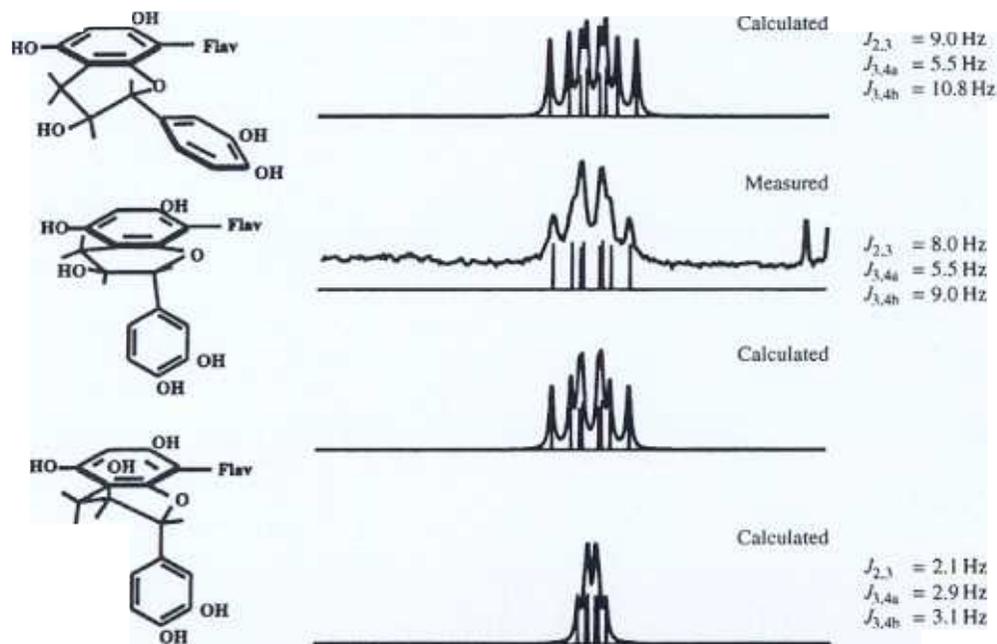


Fig. 8 PCPMR lineshape analyses of the H-3_F proton in **2** when recorded in D₂O.

no NOE correlations between the B- and E-rings were observed to support that conclusion. However, all data support a conformation as represented in the stereoview shown in Fig. 7 for 1 in water.

The long-range COSY spectrum of 2 in D₂O did not show correlation between H-4_C and H-6_D indicating that the torsion angle H4-C4-D8-D7 must lie close to either 0° or 180°. In addition the strong upfield shift of the protons of the B- and E-rings indicates that these two rings lie close to each other requiring an axial orientation of the E-ring. Lineshape analysis of H-3_F of 2 when measured in D₂O (Fig. 8) also suggests that the heterocyclic ring in the terminal catechin unit must take largely a skewed-boat conformation although there is more half-chair character to the coupling constants than was observed in 1. The coupling constants observed for this ring may be representative of a time-average of multiple conformations, but it does not appear possible to account for the lineshape of H-3_F by an averaging of E- and A-conformers.

Experimental

Douglas-fir bark was extracted with acetone-water 7:3 (v/v) at room temperature for 3 days and the concentrated extract solution was extracted with diethyl ether (4 times) to recover 3.0% of an ether extract. Chromatography on Sephadex LH-20 gave 2.5% yield of (2R,3S)-dihydroquercetin on the basis of the bark dry weight. Compounds 1 and 2 were synthesized from dihydroquercetin by reaction with sodium borohydride and either (-)-epicatechin or (+)-catechin as a capture nucleophile.²⁶ NMR spectra were recorded using a Bruker AC-300 spectrometer fitted with inverse electronics. The ¹H-¹H long-range COSY and NOESY spectra were symmetrized prior to plotting. When measured in D₂O, a small amount of acetone (at δ_H 2.04) was used as the internal standard. Stereoviews in Figs. 3 and 7 were generated using Chem3D.²⁷

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