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# Conformation and Complexation of Tannins: NMR Spectra and Molecular Search Modeling of Flavan-3-ols†

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**Studies of flavan-3-ols in their biologically significant phenolic form show that both H-6 and C-6 resonances are downfield from H-8 and C-8. Therefore, assignments for the H atoms of the A-ring are inverse to those commonly reported. By contrast, in the methyl ether and methyl ether acetate derivatives, both H-8 and C-8 are downfield from H-6 and C-6 and assignments commonly reported for C-6 and C-8 are incorrect. The assignments commonly reported for the peracetate derivatives are correct. In contrast to results observed for dimeric flavans, solvent effects on chemical shifts and coupling constants are small in monomeric flavan derivatives. The small heterocyclic ring $J_{2,3}$ coupling constants of 2,3-*cis*-flavans can be defined by lineshape analysis of H-3. These results provide the first evidence for the conformations of the common 2,3-*cis*-flavans in solution. With the exception of compounds carrying a bulky acetate at C-3, a GMMX global search protocol provides reasonable predictions of heterocyclic ring coupling constants.**

KEY WORDS NMR; $^1$H NMR; $^{13}$C NMR; conformational analysis; flavonoids; condensed tannins

## INTRODUCTION

The belief that we can better understand the biological significance of plant polyphenols (tannins) by learning more about their complexation with other biopolymers has led to substantial investments in improving our understanding of the interaction of polyflavanoids with proteins.[1–5] Much of this effort has been directed to developing a more detailed understanding of the conformational preferences and flexibility of polyflavanoids[6–9] and their interaction with polypeptides.[5,10–12] When combined, NMR and computational chemistry methods offer powerful insights into the nature of polyphenol–protein complexation.[12] To apply these methods successfully to the understanding of the relationships between polyflavanoid conformation and complexation with other polymers, it is essential that NMR resonances for proton and carbon atoms are accurately assigned and that conformational influences on heterocyclic ring coupling constants are well understood. Similarly, it is important that computer-based conformational analyses adequately represent the properties of these compounds in biologically significant environments. Although solid-state crystal structure data are helpful,[13–21] conformations found in the solid state often do not reflect those in solution. It is important to note that neither the NMR spectra nor the crystal structure of the most common flavan-3-ol, (+)-catechin (**1**), have been adequately defined.

Stimulated by the observation of a 2,3-diaxial conformation in the crystal structure of penta-*O*-acetyl-(+)-catechin (**2**), in contrast to NMR coupling constants ($J_{2,3}$ = 6.3 Hz) consistent with a rapid flexing of the heterocyclic ring in solution,[15] Porter *et al.*[20] expanded on the premise that observed $J_{2,3}$ coupling constants could be accounted for by a rapid interchange of the heterocyclic ring between equatorial (**E**) and axial (**A**) (B-ring) conformations in solution (Fig. 1). Whereas various proportions of **E** and **A** conformers were proposed to account for the heterocyclic ring coupling constants, the relative total steric energies predicted by MM2 computations were not consistent with the distributions of these conformers as predicted from $J_{2,3}$ coupling constants.[8,20] Fronczek *et al.*,[21] using the molecular dynamics in Sybyl-4.1c, came reasonably close to predicting the observed $J_{2,3}$ for tetra-*O*-methyl-(+)-catechin (**3**). More recently, Tobiason and Hemingway,[22] using the GMMX global molecular search

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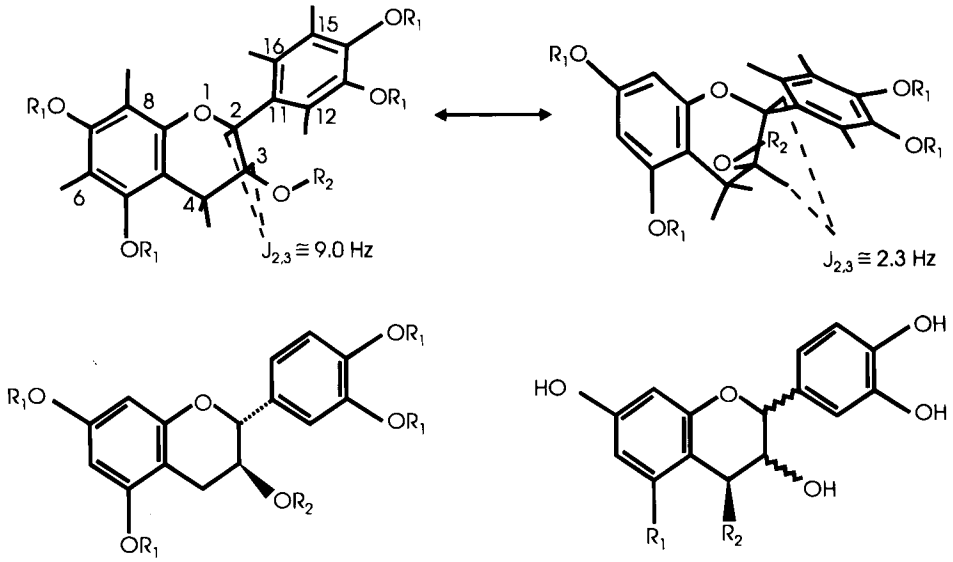


(1) $R_1 = H$; $R_2 = H$

(2) $R_1 = Ac$; $R_2 = Ac$

(3) $R_1 = Me$; $R_2 = H$

(6) $R_1 = Me$; $R_2 = Ac$

(4) $R_1 = OH$; $R_2 = H$; 2·····; 3·····

(5) $R_1 = H$; $R_2 = H$; 2◄; 3◄

(7) $R_1 = OH$; $R_2$ = phloroglucinol; 2·····; 3·····

**Figure 1.** Structures and **A** and **E** conformations of flavan-3-ols studied.

protocol[23] with PC-MODEL,[24] accurately predicted the coupling constants of **3** by computing Boltzmann-averaged torsional angles and coupling constants from ensembles of approximately 400 unique conformers found within a 3 kcal (1 kcal = 4.184 kJ) window for this molecule. These results support the Porter *et al.* hypothesis[20] that one can account for the heterocyclic ring coupling constants through a fast flip between **E** and **A** conformations. In contrast to molecular mechanics comparisons of relative energies of the two conformations,[7,8] computed coupling constants obtained by Boltzmann averaging the ensemble of conformers found in GMMX global search methods closely match observed values.[22]

Conformational properties of methyl ether or methyl ether acetate derivatives of dimeric flavans, including the proportions of the two preferred rotamers and, importantly, the heterocyclic ring conformations for the upper and lower flavan units in dimeric proanthocyanidins, can be extracted from NMR data.[25] The question now centers on whether or not it will be possible to use molecular search methods to predict the conformations of the biologically significant, free phenolic forms of these flavonoids. Our criterion for success centers on the ability of the computational method to predict accurately heterocyclic ring coupling constants observed in NMR experiments. At the same time, it is necessary to assign definitively the proton and carbon resonances. We studied (+)-catechin (**1**), (−)-epicatechin (**4**) and (+)-*ent*-epifisetinidol (**5**) to represent both 2,3-*trans* and 2,3-*cis* stereochemistry and 5,7-dihydroxy- and 7-hydroxyflavans. The latter compound was also chosen because the crystal structure and molecular mechanics using a variety of force fields have been reported previously.[19] In addition, we examined the methyl ether acetate (**6**), methyl ether (**3**) and peracetate (**2**) derivatives of catechin; the crystal structure of the last two compounds had been defined. As a start toward analysis of dimeric proanthocyanidins, we also studied (−)-epicatechin-4β-phloroglucinol (**7**) as a model for the most abundant condensed tannins in nature.

## RESULTS AND DISCUSSION

### Assignment of A-ring proton and carbon NMR resonances for (+)-catechin derivatives

Despite decades of research on the NMR spectra of these compounds,[26–29] significant problems remain in assigning the A-ring proton and carbon resonances of flavan-3-ols and their derivatives and in obtaining a satisfactory interpretation of the heterocyclic ring coupling constants, especially for the biologically significant free phenolic forms of these compounds. Recent work by Shen *et al.*[30] using COLOC and HMBC correlation experiments on **1** and other flavonoids recorded in DMSO-$d_6$ indicated that the higher field, *meta*-coupled Ar–H doublet (5.72 ppm, 2.2 Hz) was correlated with C-9 (155.3 ppm) and that the C-9 carbon signal in turn was correlated with the H-2 doublet (4.51 ppm, 7.3 Hz). This, together with a HETCORR experiment showing correlation of the higher field proton with the higher field carbon resonance, proved that both H-8 and C-8 resonate at higher field than H-6 and C-6, respectively. Significantly, these assignments are inverse to assignments made for the residual A-ring protons in methyl ether acetate (**5**) derivatives of brominated flavan-3-ols where the position of bromination has been established

by x-ray crystallographic methods and results reported for dimeric profisetinidins.[25,29] Similarly to Shen *et al.* work,[30] Pedersen *et al.*[31] made assignments for the position 6 and 8 proton and carbon resonances in anthocyanins. Their results suggested that the relative chemical shifts of H-6 and H-8 were dependent on the solvent used. Study of pelargonidin-3-glucopyranoside in acidified MeOH-$d_4$ showed an NOE between H-12 and H-16 of the B-ring to the H-8 at 253°, suggesting that the H-8 was downfield from the resonance for H-6.

The A-ring protons of catechin (**1**) appear as a pair of *meta*-coupled (about 2 Hz) doublets in the region of 5.7–6.1 ppm depending on the solvent (Table 1). Long-range COSY experiments on **1** in MeOH-$d_4$ showed the *meta* correlation between H-6 and H-8 together with weak cross peaks between the H-4 protons and the high field (later to be shown to be the H-8) proton. No correlation was observed between the heterocyclic ring H-2 and H-8 across the pyran oxygen. HETCORR experiments on **1** showed that the high-field proton resonance was always correlated with the high-field carbon signal (Table 1) in all solvents examined.

Our attempts with COLOC experiments on **1** in DMSO-$d_6$ optimized for 8 or 10 Hz $J_{CH}$ coupling did not show selective correlations between H-2 and any A-ring *C*-OR carbon unless the refocusing delay was unusually long. There was generally a strong cross peak showing correlation between the high field A-ring *C*-OR resonance and the high field A-ring proton as described by Shen *et al.*[30] However, when optimized for a $^{1r}J_{CH}$ coupling of 5 Hz (Fig. 2), a clear cross peak was observed between the H-2 doublet at 4.56 ppm with $J_{2,3} = 7.0$ Hz and the high field A-ring *C*-OR resonance at 156.33 ppm requiring assignment of this carbon resonance to C-9 (Fig. 2). This experiment also showed correlations of (i) the carbon signal at 156.33 ppm with the high-field Ar-H proton at 5.72 ppm and (ii) the lower field A-ring proton with the two Ar *C*-OR resonances at 157.01 and 157.03 ppm that must be assigned to C-5 and C-7. Therefore, although the COLOC experiment had to be optimized at 5 Hz rather than 8 Hz, our results were similar to those described by Shen *et al.*,[30] and in the phenols H-8 and C-8 must be assigned to the higher field resonances in DMSO-$d_6$.

A series of COLOC experiments was also conducted on **1** in MeOH-$d_4$ (Fig. 2). When optimized for $^{1r}J_{CH} =$ 5 Hz, no correlation through the pyran oxygen between the H-2 doublet at 4.57 ppm with $J_{2,3} = 7.4$ Hz and

**Table 1. A-ring proton and carbon resonances of (+)-catechin (1) in various solvents (chemical shifts, ppm)**

| Atom | DMSO | MeOH | Acetone | Dioxane | $D_2O$[a] |
|---|---|---|---|---|---|
| H-6 | 5.83 | 5.94 | 6.03 | 5.78 | 6.00 |
| H-8 | 5.72 | 5.86 | 5.88 | 5.78 | 5.91 |
| C-5 | 157.01 | 157.56 | 157.18 | 156.84 | 156.34 |
| C-6 | 96.70 | 96.40 | 96.16 | 95.70 | 96.69 |
| C-7 | 157.03 | 157.81 | 157.70 | 157.23 | 156.34 |
| C-8 | 95.64 | 95.60 | 95.43 | 95.18 | 95.86 |
| C-9 | 156.32 | 156.92 | 156.85 | 156.66 | 155.99 |
| C-10 | 100.85 | 100.90 | 100.61 | 100.51 | 101.37 |

[a] $D_2O$ contains about 20% MeOH-$d_4$.

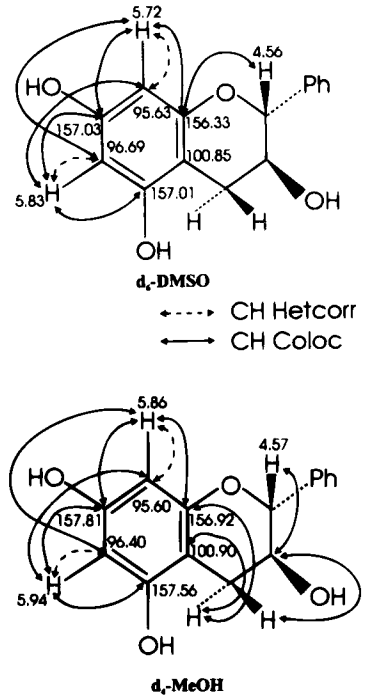


**Figure 2.** H–C correlations for (+)-catechin (**1**) in DMSO-$d_6$ and MeOH-$d_4$.

C-9 was observed. Cross peaks were observed between H-2 and C-11, C-12 and C-16 of the B-ring and to C-3 of the heterocyclic ring. Also, the H-4$\beta$ at 2.51 ppm showed correlation with C-3 of the heterocyclic ring and C-10 of the A-ring. However, in contrast to the HETCORR experiment that showed the higher field proton (H-6 or H-8) correlated with the higher field carbon (Ar-H), these cross peaks were reversed in the COLOC spectrum. The higher field proton at 5.86 ppm with $J = 2$ Hz showed cross peaks with two widely separated A-ring quaternaries at 157.81 and 156.92 ppm as well as the Ar-H carbon at 96.40 ppm, whereas the lower field proton at 5.94 ppm showed cross peaks with the two A-ring quaternaries at 157.81 and 157.56 ppm and a strong cross peak with the Ar-H carbon at 95.60 ppm. Similar results were obtained when the COLOC experiment was optimized for a $^{1r}J_{CH}$ of 10 Hz; again, no correlation between the H-2 and C-9 was observed, but there was a cross peak between H-4$\alpha$ at 2.85 ppm and the high-field A-ring quaternary at 156.92 ppm (shown to be C-9 from the COLOC experiment) in addition to a strong cross peak to C-10 at 100.90 ppm.

Hence assignments of the C-5, C-7 and C-9 of the A-ring become especially significant. Our results on catechin (**1**) in DMSO-$d_6$ differed somewhat from those of Shen *et al.*[30] because we observed carbon signals at 157.01, 157.03 and 156.33 ppm for the A-ring *C*-OR carbons C-5, C-7 and C-9, but the relative chemical shift order of the signals is the same. In MeOH-$d_4$, the high field Ar-H doublet at 5.86 ppm shows cross peaks to this high-field signal at 156.92 and also to the lowest field A-ring quaternary at 157.81 ppm. In contrast, the lower field Ar-H doublet at 5.94 ppm is correlated to the two low-field A-ring quaternaries at 157.56 and 157.81 ppm. Therefore, assignment of the high-field A-ring *C*-OR to C-9 requires assignment of the H-6 at

5.94 and H-8 to 5.86 ppm, consistent with the relative order of these signals in DMSO.

In contrast to the assignments established for the A-ring protons of the free phenol **1**, reported assignments for the H-6 and H-8 protons in methyl ether and methyl ether acetate derivatives of 5,7-dihydroxyflavans such as **3** or **6** have invariably assigned H-6 to the higher and H-8 to the lower field resonance of the pair of *meta*-coupled Ar-H doublets.[29] These assignments are based on residual proton resonances of 6- and 8-brominated catechin and epicatechin peracetates and methyl ether acetate derivatives[29] with x-ray crystallographic proof of the structures of the methyl ether acetate derivatives of 8-bromo-(+)-catechin[16] and 6-bromo-(−)-epicatechin.[14]

In an attempt to verify these assignments, NOESY and NOE difference experiments were carried out on the methyl ether (**3**) and the methyl ether acetate (**5**) derivatives of (+)-catechin. NOESY experiments on **3** showed that the high-field methoxyl at 3.76 ppm gave a strong cross peak correlated with the downfield Ar-H at 6.14 ppm with possibly some broadening into the region of the Ar-H at 6.11 ppm (Fig. 3). By contrast, the methoxyl at 3.81 ppm showed a tighter cross peak with the higher field Ar-H. Similarly, and in marked contrast to the results obtained on methyl ether acetate derivatives of dimeric profisetinidins,[25] an NOE difference experiment showed that irradiation of the higher field methoxyl at 3.76 ppm resulted in enhancement of the lower field Ar-H doublet at 6.14 ppm, whereas irradiation of the methoxyl at 3.81 ppm resulted in the enhancement of the doublet at 6.11 ppm nearly exclusively. In one NOE difference experiment (with $D2 = 0.4$ s), irradiation of the methoxyl at 3.81 ppm showed enhancement of both the Ar-H protons, but the enhancement of the signal at 6.11 ppm was much stronger than at 6.14 ppm. Irradiation of the methoxyl at 3.76 ppm resulted in enhancement of the proton at 6.14 ppm only. These results agree with the observation that the A-ring methoxyls of tetra-*O*-methyl-(+)-catechin lie in the plane of the A-ring and do not rotate freely.[8,9]

Because conclusions from these NOE experiments were equivocal, COLOC and HETCORR experiments (Fig. 3) were carried out. The HETCORR experiment showed that the higher field proton at 6.11 ppm and the higher field carbon at 91.93 ppm were correlated, and also the proton at 6.14 ppm and the carbon at 93.05 ppm were correlated (Table 2). As was seen in the

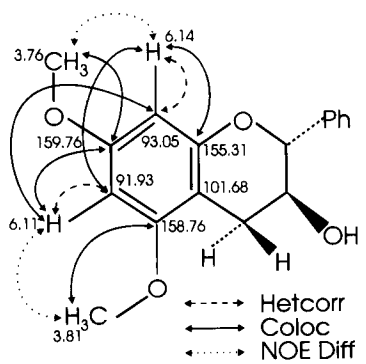


**Figure 3.** Correlations for the A-ring of tetra-*O*-methyl catechin (**3**).

**Table 2. Proton and carbon resonance assignments for the A-ring in catechin derivatives (chemical shifts, ppm)**

| Atom | Methyl ether ($CDCl_3$) | Methyl ether acetate ($CDCl_3$) | Peracetate $CDCl_3$ | Peracetate Benzene-$d_6$ |
|---|---|---|---|---|
| H-6 | 6.11 | 6.10 | 6.52 | 6.76 |
| H-8 | 6.14 | 6.17 | 6.59 | 6.87 |
| C-5 | 158.76 | 158.56 | 149.42 | 150.26 |
| C-6 | 91.93 | 91.82 | 108.74 | 109.16 |
| C-7 | 159.76 | 159.82 | 149.86 | 150.78 |
| C-8 | 93.05 | 93.02 | 107.64 | 107.97 |
| C-9 | 155.31 | 154.81 | 154.36 | 155.15 |
| C-10 | 101.68 | 100.71 | 110.15 | 110.85 |

COLOC experiment of the phenol **1**, when the experiment was optimized for a $^{1r}J_{CH}$ of either 5, 8 or 10 Hz, there were strong cross peaks between the A-ring protons and the unsubstituted C-6 and C-8 carbons of the A-ring with correlations reversed from those seen in the HETCORR experiment. The A-ring *C*-OR signals from C-5 and C-7 were defined by strong cross peaks between the methoxyl protons at 3.76 ppm and the lowest field A-ring *C*-OR at 159.76 ppm and the proton at 3.81 ppm was correlated with the carbon at 158.76 ppm. This leaves the A-ring *C*-OR carbon resonance at 155.31 ppm that must be assigned to C-9. Consideration of the C-1 and *meta* substituent effects of methoxyl *vs.* hydroxyl substitution is in accord with these conclusions.[28,32,33] The two A-ring proton signals were also correlated with substituted A-ring carbon resonances but these cross peaks differed from those seen in the COLOC spectrum of the phenol **1**. The higher field proton at 6.11 ppm showed a cross peak with the lowest field A-ring *C*-OR carbon at 159.76 ppm but no correlation to either of the other two A-ring *C*-OR resonances. Similarly, the lower field Ar-H proton resonance at 6.14 ppm showed a strong cross peak with the carbon resonance at 155.31 ppm that must be assigned to the C-9 carbon as described above. This experiment shows that H-6 must be assigned the signal at 6.11 and H-8 the signal at 6.14 ppm, consistent with the proton assignments summarized by Kolodziej.[29] The correlation to C-9 and results of the HETCORR experiment shows that the C-6 must be assigned to the resonance at 91.93 ppm and C-8 to the resonance at 93.05 ppm. These assignments for the C-6 and C-8 are inverse to those commonly cited in the literature as summarized by Agrawal *et al.*[32]

Results for the tetra-*O*-methyl-3-*O*-acetyl-(+)-catechin derivative **6** were similar to those obtained from the methyl ether **3** (Table 2). In **6**, the 7- and 5-methoxyl protons were nearly coincident at about 3.77 ppm, but they could be defined by an NOE difference experiment through irradiation of the A-ring Ar-H protons at 6.10 and 6.17 ppm. This experiment showed that the higher field A-ring methoxyl signal at 3.769 ppm was correlated with the A-ring proton at 6.17 ppm and that the lower field methoxyl at 3.774 ppm was correlated with the A-ring proton at 6.10 ppm. A HETCORR experiment showed that the higher field A-ring proton at 6.10 ppm was correlated with the high-field carbon at 91.82

ppm, and the lower field proton at 6.17 ppm was correlated with the carbon signal at 93.02 ppm. As was observed in a COLOC experiment optimized for a $^{1r}J_{CH}$ of 5 Hz for **3**, cross peaks were observed between the A-ring methoxyl protons and the A-ring *C*-OR resonances at 159.82 and 158.56 ppm, allowing assignment of C-9 to the signal at 154.81 ppm. In addition, the proton at 6.10 ppm was correlated with the carbon resonance at 159.81 ppm, whereas the proton at 6.17 ppm was correlated with the carbon at 154.81 ppm that must be assigned to C-9. Therefore, both H-6 and C-6 must be assigned the lower field resonances, inverse to the assignments of the C-6 and C-8 carbons summarized by Agrawal *et al.*[32]

The assignments for the position 6 and 8 proton and carbon resonances of peracetate derivatives must also be questioned. The proton spectrum of the peracetate of the catechin derivative **2** recorded in $CDCl_3$ shows the A-ring protons and unsubstituted carbon signals as well resolved, but the A-ring acetate protons are coincident. In contrast to results obtained on the methyl ether, a HETCORR experiment on **2** shows that the higher field proton at 6.52 ppm is correlated with the lower field unsubstituted A-ring carbon at 108.74 ppm and the A-ring Ar-H at 6.59 ppm is correlated with the carbon at 107.64 ppm (Table 2).

An NOE difference approach to assignments of the A-ring protons was possible because of sufficient resolution of the A-ring acetoxyls (1.695 and 1.685 ppm) when **2** is in benzene-$d_6$ (Fig. 4). Irradiation of the acetoxyl at 1.695 ppm gave weak but clear enhancement of both of the *meta*-coupled doublets at 6.76 and 6.87 ppm. By contrast, irradiation of the acetoxyl at 1.685 ppm resulted in very weak but selective enhancement of the Ar-H proton at 6.76 ppm. On this basis, we assign the proton at 6.87 ppm to H-8 and that at 6.76 ppm to H-6, consistent with the assignments summarized by Kolodziej.[29] A HETCORR experiment on **2** in benzene-$d_6$ showed the same correlations between the A-ring protons and unsubstituted carbons as was observed in $CDCl_3$ with the higher field proton at 6.76 ppm being correlated with the lower field carbon resonance at 109.16 ppm. The proton at 6.87 ppm was correlated with the carbon resonance at 107.97 ppm, showing that the carbon assignments for C-6 and C-8 would be consistent with those commonly reported.[28,32]

A series of COLOC experiments was made in an attempt to obtain further evidence for assignment of the peracetate derivatives (Fig. 4). As noted in analysis of the methyl ether A-ring *C*-OR signals, empirical chemical shift parameters for the C-1 and *meta* effects of a hydroxyl, methoxyl and acetoxyl substitution of the A-ring suggest that the chemical shift for C-9 should not be significantly affected, but we would expect substantial differences in the C-5 and C-7 carbon chemical shifts between the phenol, methyl ether and peracetate derivatives.[33] On that basis we would assign the C-9 resonance to the signal at 155.15 ppm, whereas the C-5 and C-7 resonances would be upfield at 150.26 and 150.78 ppm owing to the smaller C-1 effect of the acetoxyl substituent. When optimized for $^{1r}J_{CH}$ of 5 Hz, no correlation was seen between the H-2 of the heterocyclic ring and any A-ring *C*-OR. However, the higher field A-ring proton at 6.76 ppm showed cross peaks with the two low-field carbons at 150.26 and 150.78 ppm, whereas the lower field A-ring proton at 6.87 ppm was correlated with the A-ring *C*-OR resonances at 155.15 and 150.78 ppm. On the basis of both the NOE difference and COLOC experiments, we must assign the H-8 to 6.87 and H-6 to 6.76 ppm, and from the HETCORR experiment the corresponding C-8 and C-6 resonances at 107.97 and 109.16 ppm, respectively. Therefore, both the proton assignments summarized by Kolodziej[29] and the carbon assignments summarized by Agrawal *et al.*[32] and Foo and Porter[34] are correct.

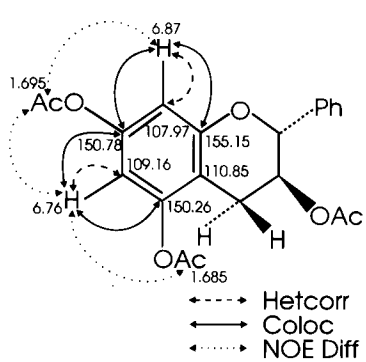


**Figure 4.** Correlations for the A-ring of the penta-acetate of the (+)-catechin **2**.

## Assignment of catechol B-ring proton and carbon resonances

The assignments of the B-ring proton resonances for the phenol **1** are typically straightforward because of the *ortho* and *meta* couplings between the H-5/H-6 and H-2/H-6, respectively, in a substituted catechol ring (Table 3). When measured in methanol-$d_4$, the H-12 doublet at 6.83 ppm with $J \approx 2.0$ Hz is well downfield from H-15 at 6.77 ppm, which appears as a distorted doublet with $J \approx 8$ Hz. The H-16 at 6.73 ppm appears as a distorted double doublet (Table 3). A HETCORR spectrum permits the assignment of the B-ring Ar-H carbon resonances as 115.28, 116.13 and 120.06 for C-12, C-15 and C-16, respectively. The resonance at 132.21 ppm is assigned to C-11 on the basis of substitution effects. The B-ring *C*-OR signals for C-13 and C-14

**Table 3. Proton and carbon resonance assignments for the B-ring in catechin derivatives (chemical shifts, ppm)**

| Atom | Phenol (MeOH-$d_4$) | Methyl ether ($CDCl_3$) | Methyl ether acetate ($CDCl_3$) | Peracetate (benzene-$d_6$) |
|---|---|---|---|---|
| H-12 | 6.83 | 6.98 | 6.89 | 7.21 |
| H-15 | 6.77 | 6.90 | 6.83 | 7.00 |
| H-16 | 6.73 | 7.01 | 6.90 | 7.05 |
| C-11 | 132.21 | 130.31 | 130.31 | 136.50 |
| C-12 | 115.28 | 110.04 | 109.64 | 122.44 |
| C-13 | 146.19 | 149.40 | 148.94 | 142.77 |
| C-14 | 146.19 | 149.40 | 148.94 | 142.84 |
| C-15 | 116.13 | 111.30 | 111.01 | 123.93 |
| C-16 | 120.06 | 119.94 | 119.19 | 124.64 |

are superimposed at 146.19 ppm. These assignments are consistent with those reported in the literature.[29,32]

Corresponding assignments for the H-12, H-15 and H-16 protons in the methyl ether derivative **3** are 6.98, 6.90 and 7.01 ppm, respectively. The downfield shift of H-16 in the methyl ether as compared with the phenol is notable. As before, the HETCORR experiment permits assignment of the C-12, C-15 and C-16 carbon resonances while the C-13 and C-14 signals are superimposed (Table 3).

A COLOC experiment on the tetra-*O*-acetyl derivative **6** optimized for $^{1r}J_{CH}$ of 5 Hz shows strong cross peaks between H-15 and C-11, H-12 and C-16 and H-16 and C-12. A weak cross peak is seen between H-15 and the B-ring *C*-OR resonance at 148.94 ppm. The methoxyls assigned to the B-ring could be verified through correlation of the two downfield methoxyls at 3.85 and 3.87 ppm with the C-13 and C-14 B-ring *C*-OR resonance at 148.94 ppm. An NOE difference spectrum then permitted assignment of the methoxyl at 3.87 ppm to that attached at C-14 because of enhancement of the H-15 with $J \approx 7$ Hz centered at 6.83 ppm. Likewise, the methoxyl at 3.85 ppm must be assigned to that attached to C-13 because of enhancement of the H-12 with a small coupling constant at 6.89 ppm.

Whereas a HETCORR experiment on the pentaacetate derivative **2** permitted assignment of the C-12, C-15 and C-16 resonances (Table 3), definition of the assignments for the nearly resolved C-13 and C-14 carbons at 142.77 and 142.83 ppm required COLOC experiments. When optimized for $^{1r}J_{CH}$ couplings at 5 and 10 Hz, cross peaks between H-12 at 7.21 ppm with the carbon at 142.77 ppm and H-15 at 7.00 ppm with the carbon at 142.83 ppm define the assignments of C-13 to the higher field carbon resonance and C-14 to that at lower field (Table 3).

Assignment of the B-ring proton and carbon resonances for *ent*-epifisetinidol (**5**) is more complicated because the H-5 of the resorcinolic A-ring must be distinguished from H-15 where both have similar couplings of about 8 Hz. When recorded in methanol-$d_4$ these two protons are readily distinguished by a HETCORR experiment that shows the low-field carbon resonance at 131.41 ppm (which must be assigned to C-5) correlated with the 7 Hz doublet at 6.87 ppm. This leaves assignment for H-15 at 6.78 ppm and for H-12 to the 2 Hz doublet at 6.97 ppm. The HETCORR experiment then shows that C-12, C-15 and C-16 are the signals at 115.32, 115.92 and 119.38 ppm, respectively. The assignment for C-11 at 132.17 ppm is also distinguished from the C-5 of the A-ring by the HETCORR experiment. The C-13 and C-14 carbon resonances are slightly resolved at 145.82 and 145.96 ppm. A COLOC experiment optimized for a $^{1r}J_{CH}$ of 5 Hz showed correlation between H-12 and the higher field B-ring *C*-OR at 145.76 ppm, whereas the distorted doublet at 6.78 ppm was correlated with the signal at 145.90 ppm.

## Heterocyclic ring assignments and coupling constants

Despite the importance of finally resolving questions centered on assignments of the A-ring proton and carbon resonances, the primary goal of this work was to examine how factors such as solvent or derivative might influence the heterocyclic ring conformation of flavan-3-ols and to evaluate whether or not observed $^3J_{HH}$ couplings could be predicted using molecular modeling methods. Earlier work with GMMX[23] using the MMX force field in PCMODEL[24] in computing the $J_{2,3}$ coupling constant of the methyl ether **3** showed excellent agreement with the coupling constant observed by NMR.[22]

Studies on the effect of variation of the solvent on coupling constants for (+)-catechin (**1**) and *ent*-epifisetinidol (**4**) showed a limited influence of the polarity of the solvent on the heterocyclic ring coupling constants (Table 4). For **1**, $J_{2,3}$ ranged from a low of 7.0 Hz in DMSO to a high of 7.8 Hz in dioxane. A small amount of methanol or acetone had to be added to make catechin dissolve in $D_2O$ where $J_{2,3}$ was 7.6 Hz.

**Table 4. Solvent effects on chemical shifts (δ, ppm) and coupling constants (*J*, Hz) for the heterocyclic ring**

| | | DMSO | | MeOH | | Acetone | | Dioxane | | $D_2O$[a] | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Compound | Atom | δ | *J* | δ | *J* | δ | *J* | δ | *J* | δ | *J* |
| (+)-Catechin | H-2 | 4.57 | 7.0 | 4.57 | 7.4 | 4.56 | 7.5 | 4.52 | 7.8 | 4.59 | 7.6 |
| | H-3 | 3.96 | (m) | 3.98 | (m) | 4.01 | (m) | 3.88 | (m) | 4.05 | (m) |
| | H-4α | 2.65 | 4.6, 16.0 | 2.85 | 5.4, 16.2 | 0.92 | 5.3, 16.9 | 2.82 | 5.4, 16.4 | 2.77 | 5.3, 16.1 |
| | H-4β | 2.39 | 7.5, 16.0 | 2.51 | 8.0, 16.2 | 0.93 | 8.4, 16.9 | 2.42 | 8.2, 16.4 | 2.44 | 8.0, 16.1 |
| | C-2 | 81.7 | | 82.8 | | 82.7 | | 82.39 | | 81.93 | |
| | C-3 | 67.5 | | 68.7 | | 68.3 | | 68.07 | | 67.71 | |
| | C-4 | 28.1 | | 28.4 | | 28.8 | | 28.39 | | 27.61 | |
| (+)-*ent*-Epifisetinidol | H-2 | 4.82 | (br-s) | 4.88 | (br-s) | 4.94 | (br-s) | 4.85 | (br-s) | 4.97 | (br-s) |
| | H-3 | 4.02 | (m) | 4.16 | (m) | 4.20 | (m) | 4.04 | (m) | 4.28 | (m) |
| | H-4α | 2.99 | 3.7, 16.0 | 3.11 | 4.2, 16.0 | 3.10 | 4.2, 16.1 | 3.03 | 4.2, 16.1 | 3.13 | 4.1, 16.4 |
| | H-4β | 2.56 | 3.2, 16.0 | 2.71 | 3.0, 16.0 | 2.72 | 3.2, 16.1 | 2.65 | 3.1, 16.1 | 2.78 | 3.5, 16.4 |
| | C-2 | 78.28 | | 80.01 | | 79.56 | | 79.39 | | 78.83 | |
| | C-3 | 65.26 | | 67.76 | | 67.22 | | 67.18 | | 66.53 | |
| | C-4 | 32.99 | | 33.98 | | 33.04 | | 33.71 | | 32.68 | |

[a] Solvent for catechin is $D_2O$ containing about 20% MeOH-$d_4$ and solvent for *ent*-epifisetinidol is $D_2O$ containing about 20%acetone-$d_6$.

If these changes in coupling constants are considered to reflect differences in proportions of **A** and **E** conformations, this would represent a change in **A**:**E** ratio from about 41:59 in DMSO to 30:70 in dioxane on the basis of the coupling constants for optimized structures of the **A** ($J_{2,3}$ = 2.61 Hz) and **E** ($J_{2,3}$ = 10.04 Hz) conformers using the MMX force field. Predicted coupling constants obtained from MM2, AM1, MNDO and PM3 force fields do not differ substantially from those obtained with MMX.[8,9] In addition, the existence of a small but significant NOE association from H-6 of the B-ring to H-4 of the heterocycle has been reported.[8] That observation lends credence to the molecular dynamics and molecular search computations that show these coupling constants must be considered as time-averaged values from rapid flexing of the heterocycle between **A** and **E** conformations. Therefore, the free phenol **1** in aqueous solution would be expected to exist in a mixture of **A** and **E** conformers at a proportion of about 33:67.

The H-2 proton in 2,3-*cis*-flavan-3-ols such as (−)-epicatechin (**4**) and *ent*-epifisetinidol appears as a broadened singlet (Table 4). Therefore, attention was directed to H-3 coupled with proton spectrum simulations using the PCPMR[35] in an attempt to define the heterocyclic ring coupling constants in these compounds (Fig. 5). The signals of the heterocyclic ring protons of epicatechin (**4**) in methanol-$d_4$ are shown expanded, together with a simulation of the H-3 proton using the $J_{3,4\alpha}$ and $J_{3,4\beta}$ data that can be measured from the spectrum. By varying the $J_{2,3}$ coupling and line broadening factor, it is possible to match closely the observed H-3 multiplet. This approach suggested the following heterocyclic ring coupling constants for (−)-epicatechin: $J_{2,3}$ = 1.6 Hz, $J_{3,4\alpha}$ = 4.5 Hz, $J_{3,4\beta}$ = 3.3 Hz and $J_{4\alpha,4\beta}$ = 16.8 Hz, a much larger coupling constant than heretofore assumed and suggestive of significant **A** conformation in (−)-epicatechin. The computed $J_{2,3}$ for (−)-epicatechin is 0.53 and about 4.5 Hz for the **E** and **A** conformers, respectively, when evaluated using the MMX force field. Computed $J_{3,4\alpha}$ and $J_{3,4\beta}$ coupling constants for the **E** conformer are 3.1 and 2.8 Hz and those for the **A** conformer are 10.9 and 5.3 Hz. The observed coupling constants could be accounted for by an average population of **A** and **E** conformers of about 20:80 on the basis of $J_{2,3}$, about 15:85 on the basis of $J_{3,4\alpha}$ and 17:83 on the basis of $J_{3,4\beta}$, respectively. Our results are in general agreement with those obtained in study of the (2*R*,3*R*)-(−)-epicatechin (**4**)[8,9] and (2*S*,3*S*)-*ent*-epifisetinidol (**5**).[19]

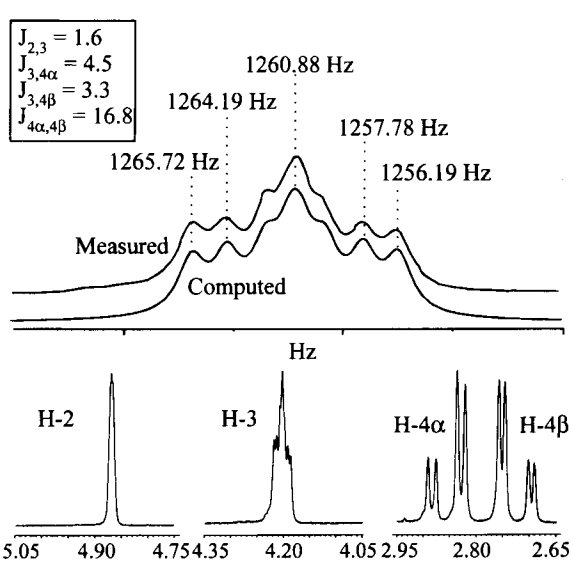


**Figure 5.** Observed and PCPMR-computed coupling constants for the heterocyclic ring of (−)-epicatechin (**4**).

To explore this approach further, similar studies were made of epicatechin-4β-phloroglucinol (**7**) (Fig. 6). Comparison of simulated and measured lineshapes for H-3 of epicatechin-4β-phloroglucinol indicated $J_{2,3}$ = 1.0 Hz and $J_{3,4}$ = 2.0 Hz, showing a much higher preference for the **E** conformer consistent with the effects of a bulky phloroglucinol substituent at C-4.

Derivatization resulted in the largest change in conformation with $J_{2,3}$ at its smallest, only 6.4 Hz for the peracetate **2** in $CDCl_3$ and at its highest, 8.4 Hz for the methyl ether **3** (Table 5). The NMR data suggest that addition of a bulky group at C-3 such as in the derivatives **2** and **5** results in a substantial preference for a 2,3-diaxial **A** conformer. In the crystal state[19] the **A** conformer is obtained.

When considering the effect of an acetyl group at C-3, AM1 predicts only small differences in the energies of the **A** and **E** conformers for the 2,3-*trans*-catechin derivative ($\Delta H_f$ = −248.24 *vs.* −248.41 kcal mol$^{-1}$), and the **A** conformer is predicted to be slightly more stable in the 2,3-*cis*-epicatechin ($\Delta H_f$ = −247.29 *vs.* −247.02 kcal mol$^{-1}$) derivative.[9] By contrast, NMR data for the methyl ether derivative **3** suggest that this compound exists in a far higher proportion as the more extended **E** conformer (Table 5).

## Conformational analyses by molecular search (GMMX) methods

The GMMX searching program[23] was used in the statistical mixed atom and internal bond coordinate mode. Studies reported here also include variation of both the hydrogen bonding and the dielectric constant. The search was first carried out with non-oxygen hydrogen atoms removed from a structure that was generated in MMX format out of the PCMODEL program.[24] When the first ensemble of conformers has been found, it is put through a reiterative process where the hydrogen atoms are removed and then again added before the re-minimization takes place until no further structures are dropped from the ensemble. Movement in all exocyclic rotating groups and all potential conformations within the pyran ring was allowed, but only structures within a relative energy ≤3 kcal mol$^{-1}$ were retained in the final ensemble since conformers having higher relative energies do not contribute to averaging NMR coupling constants. The hydrogen bonding can be switched on or off through the use of an additional $1/r$ term added to the bond dipole interactions.

The probability of each conformer within the molecular ensemble is derived using the Boltzmann equation.[22] The coupling constant is then Boltzmann averaged over

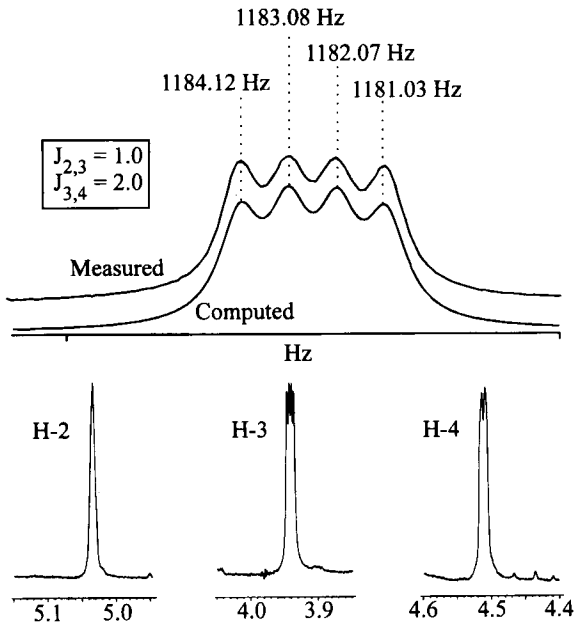


**Figure 6.** Observed and PCPMR-computed coupling constants for epicatechin-4$\beta$-phloroglucinol (**7**).

all conformers within a specified energy window using the Altona[35]-modified Karplus equation.[36] Such a Boltzmann-averaged computation of $J_{2,3}$ from the 400 or so conformers existing in a 3 kcal window provided an accurate prediction of the $J_{2,3}$ coupling constant found for tetra-*O*-methyl-(+)-catechin (**3**).[22]

To test the MMX force field and GMMX global search routine more thoroughly, we compared the coupling constants predicted from Boltzmann-averaged conformers found in GMMX searches with observed NMR heterocyclic ring coupling constants for (+)-catechin (**1**), tetra-*O*-methyl-(+)-catechin (**3**), tetra-*O*-methyl-3-*O*-acetyl-(+)-catechin (**6**), (−)-epicatechin (**4**), *ent*-epifisetinidol (**5**) and (−)-epicatechin-4$\beta$-phloroglucinol (**7**).

GMMX search studies of catechin (**1**) centered on defining the variability of predicted pyran ring coupling constants associated with changes in dielectric constant ($\varepsilon$) and with turning the hydrogen bonding function on or off (Table 6). The computed coupling constants most closely fit experimental NMR data with the dielectric constant set between 4 and 5, and the hydrogen bonding function turned off in polar solvents such as water and turned on in the non-polar solvents. In studies of **1**, all three of the computed pyran ring coupling constants agree very well with NMR data. It is also evident that there is a trend from higher to lower coupling constant values as the dielectric constant increases, which is consistent with the observed values (compared $J_{2,3}$ going from 7.8 Hz in dioxane to 7.0 Hz in DMSO) shown in Table 4. The distribution of **E** and **A** conformers obtained from a search of (+)-catechin (**1**) shows that approximately 89% of the population is in an **E** conformation and 11% in an **A** conformation, and

**Table 5. Derivative effects on chemical shifts ($\delta$, ppm) and coupling constants ($J$, Hz) of (+)-catechin**

| Atom | Methyl ether ($CDCl_3$) | | Methyl ether acetate ($CDCl_3$) | | Peracetate $CDCl_3$ | | Peracetate Benzene | |
|---|---|---|---|---|---|---|---|---|
| | $\delta$ | $J$ | $\delta$ | $J$ | $\delta$ | $J$ | $\delta$ | $J$ |
| H-2 | 4.66 | 8.4 | 5.02 | 6.8 | 5.07 | 6.3 | 4.82 | 6.6 |
| H-3 | 4.06 | (m) | 5.35 | (dd) | 5.18 | (m) | 5.21 | (m) |
| H-4$\alpha$ | 3.08 | 5.7, 16.3 | 2.91 | 5.5, 16.6 | 2.80 | 5.1, 16.8 | 2.82 | 5.2, 16.6 |
| H-4$\beta$ | 2.59 | 8.1, 16.3 | 2.67 | 6.9, 16.6 | 2.56 | 6.4, 16.8 | 2.59 | 7.0, 16.6 |
| C-2 | 81.80 | | 78.33 | | 77.66 | | 78.22 | |
| C-3 | 68.27 | | 69.08 | | 68.27 | | 68.55 | |
| C-4 | 27.64 | | 24.01 | | 23.92 | | 24.55 | |

**Table 6. Variation of GMMX-computed coupling constants with dielectric constant and hydrogen bonding function (HB) for (+)-catechin**

| | $\varepsilon$ = 1.5 | | $\varepsilon$ = 5.0 | | $\varepsilon$ = 10.0 | |
|---|---|---|---|---|---|---|
| | HB on | HB off | HB on | HB off | HB on | HB off |
| Conformers searched | 4313 | 2380 | 3192 | 3580 | 4366 | 4012 |
| Final ensemble of conformers | 316 | 222 | 451 | 443 | 500 | 462 |
| $J_{2,3}$ (Hz)[a] | 8.02 | 8.25 | 7.83 | 7.67 | 7.86 | 7.50 |
| $J_{3,4\alpha}$ (Hz)[a] | 5.22 | 5.31 | 5.11 | 5.05 | 5.15 | 4.99 |
| $J_{3,4\beta}$ (Hz)[a] | 9.68 | 9.94 | 9.46 | 9.26 | 9.51 | 9.14 |
| $E_{min}$ (kcal mol$^{-1}$) | 13.1 | 12.8 | 15.9 | 15.5 | 16.3 | 16.1 |

[a] Observed coupling constants: $J_{2,3}$ = 7.6 Hz; $J_{3,4\alpha}$ = 5.3 Hz; $J_{3,4\beta}$ = 8.0 Hz.

the width of the C-2 to C-3 torsion is narrow in both populations (Fig. 7). The tightness of the heterocyclic ring conformations can be appreciated by comparison with the distribution of conformers with different B-ring orientations predicted by this same search (Fig. 8).

Table 7 shows the GMMX search results for (−)-epicatechin (**4**) and (+)-*ent*-epifisetinidol (**5**) representing the 2,3-*cis*-flavan-3-ols, tetra-*O*-methyl-(+)-catechin (**3**) and tetra-*O*-methyl-3-acetyl-(+)-catechin (**6**) representing commonly prepared derivatives and finally epicatechin-4$\beta$-phloroglucinol (**7**) as a model for the procyanidin-based condensed tannins. Comparison of predicted and observed heterocyclic ring coupling constants shows a reasonable fit, but the results are not as closely matched as those obtained in studies of (+)-catechin. A small number of **A** conformers were found for both **4** and **5** and those were found only above a relative energy level of 2 kcal mol$^{-1}$. The (+)-catechin search showed a much broader distribution of **A** conformers starting at a relative energy level of 1.4 kcal mol$^{-1}$.

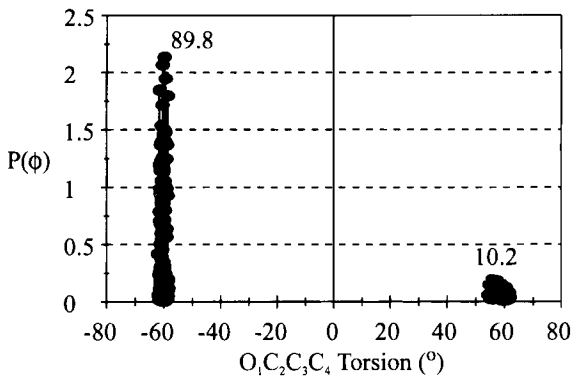


**Figure 7.** Probability distribution of heterocyclic ring conformations in the GMMX ensemble obtained for (+)-catechin.

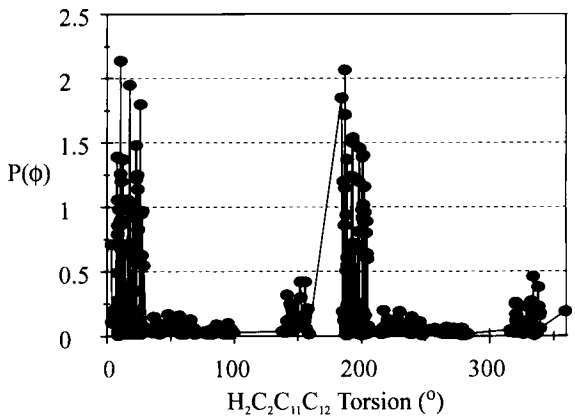


**Figure 8.** Probability distribution of B-ring orientations in the GMMX ensemble for (+)-catechin.

The Boltzmann-averaged coupling constants for epicatechin-4$\beta$-phloroglucinol (**7**) agree well with the observed coupling constants when the NMR spectrum was obtained from an acetone solution. The best fit was found when the hydrogen bonding function was turned off. With the hydrogen bonding function turned on, lower energy conformers are obtained with a hydrogen bond formed between the pyran ring 3-OH and the phoroglucinol ring 2-OH. This tends to lock the pyran ring into a rigid structure greatly favoring the **E** conformer.

An interesting problem appears in the GMMX computation for tetra-*O*-methyl-3-*O*-acetyl-(+)-catechin (**6**). The close fit between computed and observed heterocyclic ring coupling constants for tetra-*O*-methyl-(+)-catechin (**3**) is not found in **6** where a bulky acetate group is attached to the heterocyclic ring (Table 7). In studies of **6**, only a few **A** conformers are found within the relative 3 kcal mol$^{-1}$ energy window and, consequently, the computed $J_{2,3}$ coupling constant of 8.1 Hz is large compared with the experimental value of 6.8 Hz (Table 5); this, in contrast, suggests a significant proportion of **A** conformers in solution. In addition, the entire final GMMX ensemble for penta-*O*-acetyl-(+)-catechin (**2**) contained only **E** conformers with the Boltzmann-averaged coupling constants obtained being $J_{2,3} = 9.07$, $J_{3,4\alpha} = 5.44$ and $J_{3,4\beta} = 10.85$ Hz. Again, the experimental $J_{2,3} = 6.6$ Hz (Table 5) indicates a larger percentage of **A** conformers in solution and it should be recalled that this compound crystallizes in an **A** conformation.[15] Attempts to change the program input conditions and variation of input structure while leaving the MMX force field unchanged did not significantly

**Table 7. Variation of GMMX-computed coupling constants for various flavan-3-ol derivatives**

| | (−)-Epicatechin (4) | | (+)-*ent*-Epifisetinidin (5) | | Tetra-*O*-methylcatechin (3) | | (−)-Epicatechin-4$\beta$-phloroglucinol (7) | | Tetra-*O*-methyl-3-*O*-acetylcatechin (6) |
|---|---|---|---|---|---|---|---|---|---|
| | HB on | HB off | HB on | HB off | HB on | HB off | HB on | HB off | HB off |
| Conformers searched | 2087 | 3120 | 1549 | 1006 | 4957 | 7935 | 9149 | 8336 | 6164 |
| Final ensemble | 296 | 291 | 172 | 114 | 347 | 428 | 1064 | 960 | 268 |
| $J_{2,3}$ (calc.) (Hz) | 0.57 | 0.53 | 0.56 | 0.59 | 8.16 | 7.68 | 1.40 | 1.12 | 8.10 |
| $J_{2,3}$ (observed) (Hz) | 1.6 | | <1.0 | | 8.1 | | 1.0 | | 6.8 |
| $J_{3,4\alpha}$ (calc.) (Hz) | 3.14 | 3.06 | 3.14 | 3.16 | 5.20 | 5.05 | 3.21 | 2.64 | 5.08 |
| $J_{3,4\alpha}$ (observed) (Hz) | 4.5 | | 4.2 | | 5.5 | | 2.0 | | 5.5 |
| $J_{3,4\beta}$ (calc.) (Hz) | 3.08 | 3.05 | 3.00 | 3.02 | 9.88 | 9.33 | | | 9.81 |
| $J_{3,4\beta}$ (observed) (Hz) | 3.3 | | 3.0 | | 9.0 | | | | 6.9 |
| $E_{min}$ (kcal mol$^{-1}$) | 15.5 | 15.2 | 16.7 | 16.5 | 32.4 | 32.3 | 21.0 | 20.4 | 31.0 |

change these results. Work is in progress to examine changes for the torsional potentials used for the pyran ring structure and for rotation of the acetate group.

## EXPERIMENTAL

$^{13}C$ and $^{1}H$ NMR experiments were carried out using a Bruker AC-300 spectrometer (75 and 300 MHz, respectively) at 23 °C with a 5 mm dual probe in solvents as described in the Results and Discussion section. Standard pulse sequences were used for COSY, long-range COSY, NOESY, HETCORR and COLOC experiments. Condition used for NOE difference experiments are described in the Results and Discussion section. Computations were preformed on 486/66 MHz and Pentium 90-based computers.

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## REFERENCES

1. L. F. Tilstra, D. Cho, W. R. Bergmann and W. L. Mattice, in *Chemistry and Significance of Condensed Tannins*, edited by R. W. Hemingway and J. J. Karchesy, p. 335. Plenum Press, New York (1989).
2. C. A. Helfer, PhD Thesis, University of Akron, Akron, OH (1993).
3. A. E. Hagerman, in *Chemistry and Significance of Condensed Tannins*, edited by R. W. Hemingway and J. J. Karchesy, p. 323. Plenum Press, New York (1989).
4. L. G. Butler, in *Plant Polyphenols: Synthesis, Properties, Significance*, edited by R. W. Hemingway and P. E. Laks, p. 693. Plenum Press, New York (1992).
5. A. E. Hagerman and L. G. Butler, *J. Biol. Chem.* **256**, 4494 (1981).
6. V. N. Viswanadhan and W. L. Mattice, *J. Comput. Chem.* **7**, 711 (1986).
7. W. R. Bergmann, V. N. Viswanadhan and W. L. Mattice, *J. Chem. Soc., Perkin Trans. 2* 45 (1988).
8. J. P. Steynberg, E. V. Brandt, M. J. H. Hoffmann, R. W. Hemingway and D. Ferreira, in *Plant Polyphenols: Synthesis, Properties, Significance*, edited by R. W. Hemingway and P. E. Laks, p. 501. Plenum Press, New York (1992).
9. F. L. Tobiason, in *Plant Polyphenols: Synthesis, Properties, Significance*, edited by R. W. Hemingway and P. E. Laks, p. 459. Plenum Press, New York (1992).
10. L. G. Butler, in *Food Proteins*, edited by J. Kinsella and W. G. Soucie, p. 402. American Oil Chemists Society, Champaign, IL (1989).
11. L. F. Tilstra, H. Maeda and W. L. Mattice, *J. Chem. Soc., Perkin Trans 2* 6614 (1987).
12. G. Luck, H. Liao, N. J. Murray, H. R. Grimmer, E. E. Warminski, M. P. Williamson, T. H. Lilley and E. Haslam, *Phytochemistry* **37**, 357 (1994).
13. F. R. Fronczek, G. Gannuch, W. L. Mattice, F. L. Tobiason, J. L. Broeker and R. W. Hemingway, *J. Chem. Soc., Perkin Trans. 2* 1611 (1984).
14. J. C. A. Boeyens, L. Denner, H. Kolodziej, D. Ferreira and D. G. Roux, *J. Chem. Soc., Perkin Trans. 2* 301 (1986).
15. F. R. Fronczek, G. Gannuch, W. L. Mattice, R. W. Hemingway, G. Chiari, F. L. Tobiason, K. Houghlum and A. Shanafelt, *J. Chem. Soc., Perkin Trans. 2* 1383 (1985).
16. D. W. Engle, M. Hatting, H. K. L. Hundt and D. G. Roux, *J. Chem. Soc., Chem. Commun.* 695 (1978).
17. F. W. B. Einstein, E. Kiehlmann and E. K. Wolowidnyk, *Can. J. Chem.* **63**, 2176 (1985).
18. L. J. Porter, R. Y. Wong and B. G. Chan, *J. Chem. Soc., Perkin Trans. 1* 1413 (1985).
19. F. L. Tobiason, F. R. Fronczek, J. P. Steynberg, E. C. Steynberg and R. W. Hemingway, *Tetrahedron* **49**, 5927 (1993).
20. L. J. Porter, R. Y. Wong, M. Benson, B. G. Chan, V. N. Viswanadhan, R. E. Gandour and W. L. Mattice, *J. Chem. Res.* (*S*) 86, (*M*) 830 (1986).
21. F. R. Fronczek, R. W. Hemingway, G. W. McGraw, J. P. Steynberg, C. A. Helfer and W. L. Mattice, *Biopolymers* **33**, 275 (1993).
22. F. L. Tobiason and R. W. Hemingway, *Tetrahedron Lett.* **35**, 2137 (1994).
23. *GMMX Global Searching Program, Version 1*. Serena Software, Bloomington, IN.
24. *PCMODEL Program, 386 or Windows Version*. Serena Software, Bloomington, IN.
25. J. P. Steynberg, E. V. Brandt, D. Ferreira, C. A. Helfer, W. L. Mattice, D. Gornik and R. W. Hemingway, *Magn. Reson. Chem.* **33**, 611 (1995).
26. K. Weinges, K. Goritz and F. Nader, *Liebigs Ann. Chem.* **715**, 164 (1968).
27. J. W. Clark-Lewis, *Aust. J. Chem.* **17**, 632 (1964).
28. P. K. Agrawal (Ed.), *Carbon-13 NMR of Flavonoids*. Elsevier, Amsterdam (1989).
29. H. Kolodziej, in *Plant Polyphenols: Synthesis, Properties, Significance*, edited by R. W. Hemingway and P. E. Laks, pp. 295–319. Plenum Press, New York (1992).
30. C.-C. Shen, Y.-S. Chang and L.-K. Ho, *Phytochemistry* **34**, 843 (1993).
31. A. T. Pedersen, O. M. Andersen, D. W. Aksnes and W. Nerdal, *Magn. Reson. Chem.* **31**, 972 (1993).
32. P. K. Agrawal, M. C. Bansal, L. J. Porter and L. Y. Foo, in *Carbon-13 NMR of Flavanoids*, edited by P. K. Agrawal, pp. 432–496. Elsevier, Amsterdam (1989).
33. F. W. Wehrli and T. Wirthlin, *Interpretation of Carbon-13 NMR Spectra*, pp. 45–47. Heyden, London (1978).
34. L. Y. Foo and L. J. Porter, *J. Chem. Soc., Perkin Trans. 1* 1535 (1983).
35. *PCPMR Spectrum Simulation Program, Version 1*. Serena Software, Bloomington, IN.
36. C. A. G. Haasnoot, F. A. A. M. DeLeeuw and C. Altona, *Tetrahedron* **36**, 2783 (1980).
37. M. Karplus, *J. Am. Chem. Soc.* **85**, 2870 (1963).