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# Conformational Analysis of Oligomeric Flavanoids

## 2*—Methyl Ether Acetate Derivatives of Profisetinidins

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**The profisetinidins are the most important polyflavanoids of commerce, making up the major constituents of wattle and quebracho tannins. Even within the dimeric profisetinidins, substantial complexity exists because of stereo-, regio-, rotational and conformational isomers. Definition of the stereochemistry of the upper and lower flavan units, the location of the interflavanoid bond, the conformation of the heterocyclic rings in the upper and lower flavan units and the conformations of major and minor rotational isomers in a series of methyl ether acetate derivatives of dimeric profisetinidin diastereomers is possible by application of COSY and NOE experiments. All compounds studied were present in two rotameric forms with the more compact conformation favored. The heterocyclic ring in the upper chain extender unit was predominantly in an E-conformation (i.e. half-chair in *2R,3S* isomers and a 'reverse' half-chair in the *2S,3R* isomers). The heterocyclic ring conformation of the terminal catechin unit, although mostly in an E-conformation, was often represented by significant proportions of A-conformers.**

KEY WORDS NMR; $^1$H NMR; condensed tannins; profisetinidins; stereochemistry; conformation; rotational isomers

## INTRODUCTION

Polymeric proanthocyanidins (*syn* condensed tannins) constitute the second (after lignins) most abundant class of natural phenolic compounds. The past 15 years have seen remarkable growth in our understanding of the basic structure of these compounds.[1,2] Essentially all of their biological significance (protection of plants from insects, diseases and herbivores) and most of the current (leather manufacture) and also most promising new uses (pharmaceuticals or wood preservatives) rest on their complexation with other biopolymers (proteins or carbohydrates) or metal ions.[3,4] Therefore, increasing attention is now being directed to understanding their conformation[5–8] (and conformational flexibility)[9,10] in order to explain their biological activity and to provide a basis for further development of uses for these renewable phenolic compounds.

Although the conformational properties of the free phenolic forms[5,11] of these compounds are most important to their biological significance, in many instances and notably among the profisetinidins, it is often necessary to work with their methyl ether acetate derivatives to obtain suitably pure compounds for NMR spectral analysis. The conformational properties of these derivatives are of interest in their own right because, even at the dimeric level, they exhibit substantial complexity due to variations in the populations of rotational and conformational isomers that, until now, has not been well defined. To establish a foundation from which to build more information on the conformations of polyflavanoids, we elected first to define the conformational properties of the methyl ether acetate derivatives.

The $^1$H NMR spectra of methyl ether acetate derivatives of dimeric profisetinidins typically display two overlapped spectra due to the presence of restricted rotation about the interflavan bond.[12,13] With only a few exceptions where only one rotational isomer predominates,[14] unequivocal assignment of the conformational structures of these rotamers has not been made. Foo and Porter[15] examined the peracetate derivatives of a similar series of 3,5,7,3′,4′,pentahydroxyflavan (procyanidin) dimers and, although significant progress was made, proof of rotamer conformations were based on assumptions about shielding effects. Kolodziej,[16] in a recent thorough review of the $^1$H NMR spectral properties of the peracetate derivatives of oligomeric procyanidins, also described rotational and conformational isomerism in these compounds but definitive evidence for the conformations of different rotational isomers has not yet been obtained. On an NMR time-scale, these rotational isomers are most evident in methyl ether acetate derivatives where restricted rotation is enhanced by the presence of methoxyl and acetoxyl functions.

In this paper, the rotational isomers are designated either (+) or (−) on the basis of the sign of A(10)—C(4)—D(6- or 8)—D(7) dihedral angle to be consistent with earlier molecular modeling work by Mattice and co-workers.[17,18] Therefore, the (4,8) linked compounds

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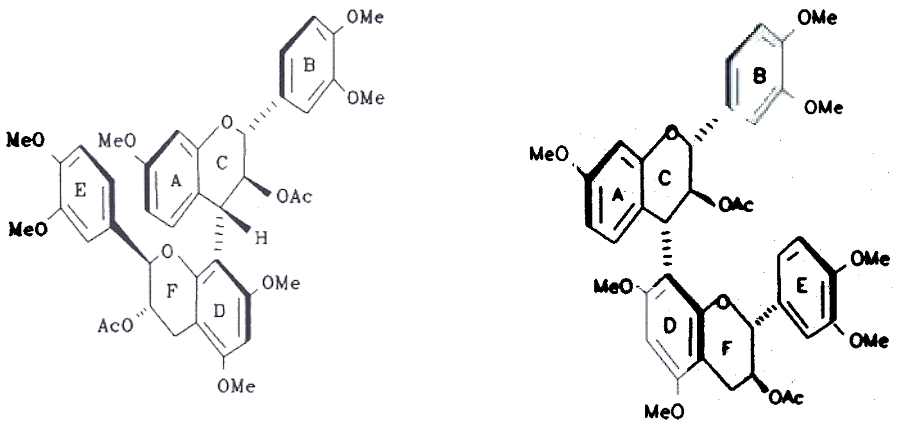
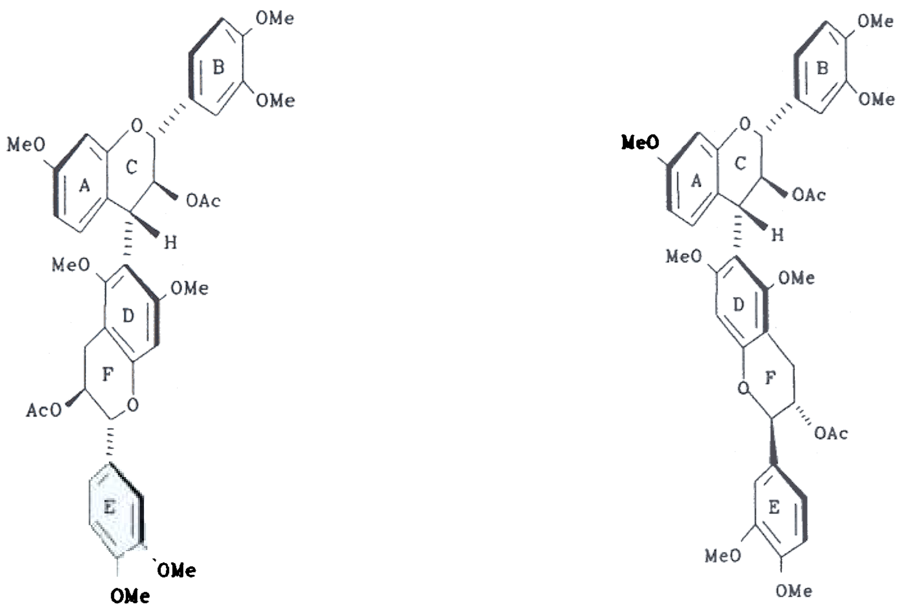
in which the E-ring of the lower unit extends out away from the A- and C-ring plane are designated (+) and those in which the E-ring is back behind the A- and C-ring plane are designated (−) rotamers (Fig. 1). For the (4,6) linked compounds, the conformations in which the A(10)—C(4)—D(6)—D(7) dihedral angle is (−) correspond to the rotamers in which the pyran oxygen of the F-ring is out away from, and when (+) back behind, the plane of the interflavanoid bond.

The conformation of these compounds is further complicated by flexing of the pyran C- and F-rings (depicted for the C-ring in Fig. 2), resulting in mixtures of conformers in which the B/E-ring is in either an axial (A-conformer) or equatorial (E-conformer) orientation.[9,11,19] Early evidence for this conformational flexibility (A/E flip of the heterocyclic ring conformation) came from the observation of a diaxial orientation of the catechol B-ring at C-2 and the acetyl group at C-3 in the crystal form of penta-*O*-acetylcatechin,[20] and the observation that $J_{2,3}$ and $J_{3,4}$ coupling constants of a wide range of flavan derivatives do not correspond to those that would be expected of either an approximate half-chair or a 'reverse' half-chair conformations of the heterocyclic ring.[19] Molecular mechanics (MM2 and MMX force fields) studies of a number of catechin and epicatechin derivatives suggest small (about 1–2 kcal $\text{mol}^{-1}$; 1 kcal = 4.184 kJ) total steric energy differences between A- and E-conformers.[10,11] Further evidence for A/E flip of the heterocyclic ring conformation was obtained from molecular dynamics analyses of tetra-*O*-methylcatechin.[9]

Porter *et al.*[19] examined the heterocyclic ring coupling constants of a series of catechin and epicatechin derivatives and demonstrated that these coupling con-

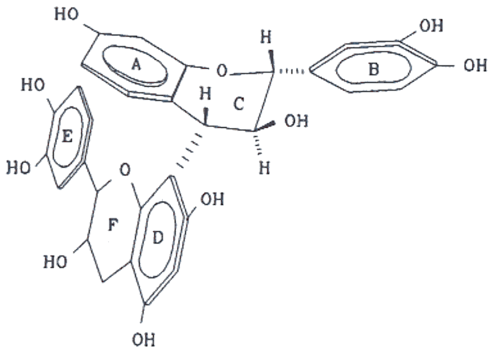


A(10) - C(4) - D(8) - D(7) = (-)        A(10) -C(4) - D(8) - D(7) = (+)

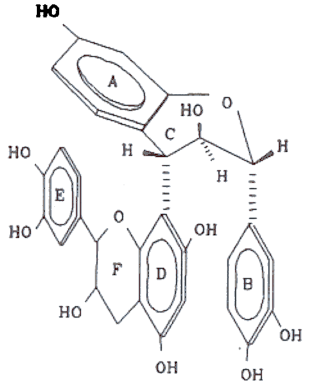


A(10) - C(4) - D(6) - D(7) = (-)        A(10) - C(4) - D(6) - D(7) = (+)

**Figure 1.** Designation of (−) and (+) rotamers in (4,8) and (4,6) linked dimers based on A(10)—C(4)—D(8) or D(6)—D(7) dihedral angles.



*E- Conformer*



*A-Conformer*

**Figure 2.** A- and E-conformations for the heterocyclic C-ring in (2*R*,3*S*)-flavans.

stants could be predicted from Haasnoot *et al.*'s modification of the Karplus equation,[21] if one assumed that the observed coupling constant was related to the mole fraction average of the compound in either the A- or E-conformation. The presence of the methoxyl and acetoxyl groups magnifies effects of both rotational and A- and E-conformational isomerism.

In this study, we examined the conformations of the heptamethyl ether diacetate derivatives of six of the eight possible 2,3-*trans*-profisetinidin dimers based on catechin; fisetinidol-(4α,8)-catechin (**1**), fisetinidol-(4β,8)-catechin (**2**), fisetinidol-(4α,6)-catechin (**3**) and fisetinidol-(4β,6)-catechin (**4**), *ent*-fisetinidol-(4α,8)-catechin (**5**) and *ent*-fisetinidol-(4β,8)-catechin (**6**) (Fig. 3). These provide examples of the variations in absolute stereochemistry of the chain extender unit (2*R* or 2*S*), the stereochemistry at C-4 (3,4-*trans* or 3,4-*cis*) and the location of the interflavanoid bond (4 → 8 or 4 → 6). Studies of the NMR spectral properties of the other two diastereomers, *ent*-fisetinidol-(4α,6)-catechin (**7**) and *ent*-fisetinidol-(4β,6)-catechin (**8**), are presented in another paper concerned with interpretation of the $^{13}C$ NMR spectra of these compounds. The series discussed here was selected to represent the major variations in stereochemistry at C-4 and interflavan bond locations commonly found in commercially available plant extracts from wattle and quebracho tannins.[22–24]

## EXPERIMENTAL

The methyl ether acetate derivatives of the profisetinidin dimers **1–8** (Fig. 3) were synthesized by reaction of mollisacacidin (**9**) or *ent*-mollisacacidin (**10**) with catechin (**11**) under acid catalysis.[22] The dimeric profisetinidins (as their free phenols) were partially (or in certain instances completely) separated by column chromatography on Sephadex LH-20 by elution with ethanol and/or ethanol–water mixtures. Methylation with diazomethane and preparative silica gel TLC followed by acetylation with acetic anhydride again followed by preparative silica gel TLC afforded the pure stereo- and regioisomers.

All spectra were recorded on a Bruker AM-300 spectrometer in $CDCl_3$ at 296–298 K (*ca.* 10 mg $ml^{-1}$). The $^1H$ NMR spectra were supplemented by COSY experiments to assist in assigning the spectra (Table 1). Assignments of the conformations of the major and minor rotamers were made through a series of nuclear Overhauser effect (NOE) experiments. Assignment of the proportion of A and E-conformations of the heterocyclic C- and F-rings was made by assuming that the observed $J_{2,3}$ and $J_{3,4}$ coupling was a time-averaged mole ratio of a fast flipping between A- and E-conformers as described by Porter *et al.*[19]

Graphics for Figs 4–8 were made by 'minimizing' structures using the Alchemy-II force field, generating stereo-views and plotting the figures using Laserplot software. The structures shown are not to be considered as fully optimized; there are too many degrees of freedom and too many low-energy conformations for an MM definition of 'one or two' preferred conformers, primarily associated with rotational isomerism alone.

## RESULTS AND DISCUSSION

Application of COSY and NOE experiments has, for the first time, permitted unequivocal assignment of the conformation of the heptamethyl ether diacetate derivatives of profisetinidin dimers. The power of these experiments lies in their ability to resolve the location of the interflavanoid bond, (4,6) or (4,8), the stereochemistry of the interflavanoid bond, 4α or 4β, and which pair of signals due to rotational isomers is to be assigned to heterocyclic ring protons of the upper chain extender unit and lower terminal flavan unit.

Once pairs of signals can be accurately assigned to the upper and lower units of the dimer, it becomes possible to define which set of signals is due to which rotamer, and then its conformation can be established through NOE experiments.[14,25] The conformations for the pyran ring of the upper and lower units of the dimers in each of the rotameric forms can then be estimated by consideration of the $J_{2,3}$ and $J_{3,4}$ coupling constants through extension of the work of Porter *et al.*[19] Here, too, we shall assume that the $^3J_{HH}$ coupling constants reflect the mole fraction population of A- and E-conformers (Fig. 2).

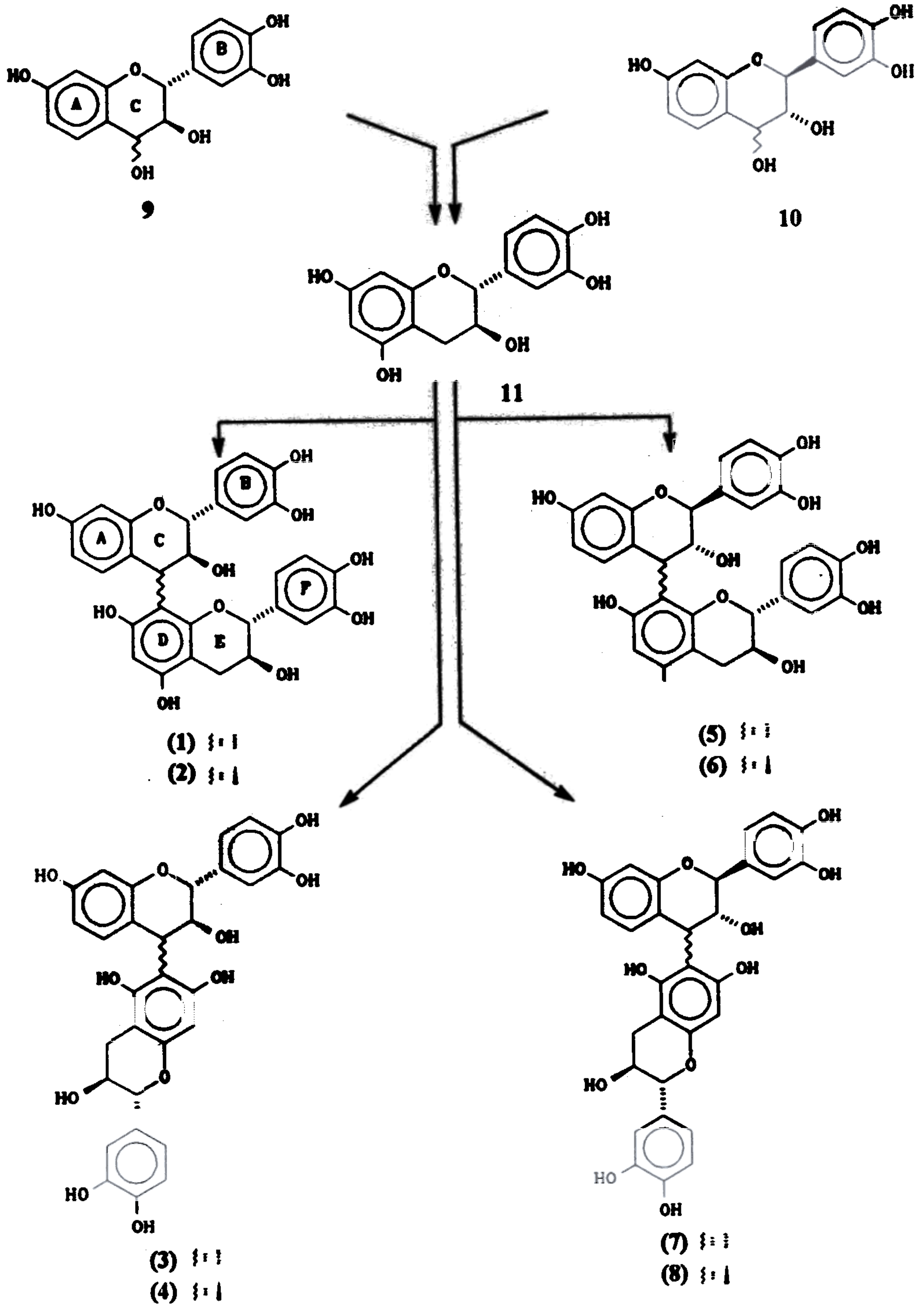


Figure 3. Synthesis of (2R,3S)- and (2S,3R)-profisetinidin dimers.

### 2*R*,3*S*,4*S*-2,3-*trans*-3,4-*trans* Stereochemistry: (4α,8) linkage

Results obtained from NMR studies of this compound represent the approaches taken with other isomers. The $^1$H NMR spectrum of the methyl ether acetate derivative of fisetinidol-(4α,8)-catechin (1) in $CDCl_3$ was especially interesting because of the appearance of mainly one rotamer (at least 80%), accompanied by small amounts of a second rotamer. Owing to the additional complexity presented by the small proportions of a second conformer, efforts were first concentrated on defining the conformation of the major rotamer.

A COSY experiment permitted the assignment of the 2-$H_C$, 3-$H_C$ and 4-$H_C$ protons of the major rotamer at $\delta$ 4.81 ppm, $J$ = 10.0 Hz; $\delta$ 6.06 ppm, $J$ = 10.0 and 10.0 Hz; and $\delta$ 4.83 ppm, $J$ = 10.0 and 1.0 Hz (Table 1). The 2-$H_F$, 3-$H_F$, 4-$H_{F\alpha}$ and 4-$H_{F\beta}$ signals were at $\delta$ 4.79, $J$ = 9.0 Hz, $\delta$ 4.87 ppm, m; $\delta$ 3.07 ppm, 16.5 and 5.5 Hz; and $\delta$ 2.58 ppm, 16.5 and 9.0 Hz. The magnitude of these coupling constants indicates that the heterocyclic rings of both the upper and lower units of the major

**Table 1.** Heterocyclic (C and F) ring $^1H$ NMR data (δ, ppm; *J*, Hz) for methyl ether acetate derivatives of profisetinidins

| Diastereomer | Rotamer | | 2-H(C) | 3-H(C) | 4-H(C) | 2-H(F) | $4\text{-H(F)}_\alpha$ | $4\text{-H(F)}_\beta$ |
|---|---|---|---|---|---|---|---|---|
| *2R* | − | δ | 4.8 | 6.06 | 4.83 | 4.79 | 3.07 | 2.58 |
| 4α, 8 | 8 | *J* | 10.0 | 10.0/10.0 | 10.0 | 9.0 | 16.5/5.5 | 16.5/9.0 |
| $CDCl_3$ | + | δ | 4.83 | 6.08 | 4.91 | 5.06 | 2.93 | 2.72 |
| | 1.5 | *J* | 10.0 | 10.0/10.0 | 10.0 | 6.5 | 16.5/5.5 | 16.5/8.0 |
| *2R* | − | δ | 5.28 | 5.56 | 4.99 | 5.11 | 2.75–2.80 | 2.58–2.62 |
| 4β, 8 | 1.0 | *J* | 10.0 | 10.0/6.5 | 6.5 | 7.0 | | |
| $CDCl_3$ | + | δ | 5.27 | 5.54 | 4.89 | 4.08 | 3.12 | 2.55 |
| | 2.0 | *J* | 10.0 | 10.06/6.5 | 6.5 | 9.0 | 16.5/5.5 | 16.5/9.0 |
| *2R* | − | δ | 4.85 | 5.95 | 4.87 | 4.84 | 3.15 | 2.65 |
| 4α, 6 | 1.0 | *J* | 10.0 | 10.0/10.0 | 10.0 | 9.0 | 16.0/5.5 | 16.0/9.0 |
| $CDCl_3$ | + | δ | 4.91 | 6.07 | 4.69 | 4.96 | 3.14 | 2.78 |
| | 2.5 | *J* | 10.0 | 10.0/10.0 | 10.0 | 8.0 | 16.0/5.5 | 16.0/8.0 |
| *2R* | − | δ | 5.22 | 5.52 | 4.71 | 4.91 | 3.15 | 2.67–2.81 |
| 4β, 6 | 1.0 | *J* | 9.0 | 9.0/6.5 | 6.5 | 9.0 | 16.0/5.5 | |
| $CDCl_3$ | + | δ | 5.28 | 5.55 | 5.02 | 5.20 | 2.67–2.81 | 2.67–2.81 |
| | 1.0 | *J* | 10.0 | 10.0/6.5 | 6.5 | 5.0 | | |
| *2S* | − | δ | 5.04 | 5.47 | 5.02 | 4.72 | 3.03 | 2.63 |
| 4α, 8 | 2.0 | *J* | 10.0 | 10.0/7.0 | 7.0 | 8.0 | 16.5/6.0 | 16.5/8.5 |
| $CDCl_3$ | + | δ | 5.29 | 5.64 | 4.86 | 4.79 | 3.08 | 2.68 |
| | 1.0 | *J* | 7.5 | 7.5/6.5 | 6.5 | 8.0 | 16.5/16.0 | 16.5/8.5 |
| *2S* | − | δ | 4.88 | 5.99 | 4.92 | 5.08 | 2.89 | 2.71 |
| 4β, 8 | 1.0 | *J* | 10.0 | 10.0/10.0 | 10.0 | 6.0 | 16.5/5.5 | 16.5/6.0 |
| $CDCl_3$ | + | δ | 4.78 | 5.88 | 4.81 | 4.40 | 2.85 | 2.55 |
| | 1.1 | *J* | 10.0 | 10.0/10.0 | 10.0 | 7.0 | 16.5/5.5 | 16.5/7.0 |

rotamer were both in approximate half-chair E-conformations.

To assign the methoxyl signals, and hence the rotational conformation of the major isomer, it was first necessary to define the Ar-H signals of the A- and D-rings through a COSY experiment. This spectrum showed the *meta*-coupled ($J = 2.5$ Hz) doublet for 8-$H_A$ at δ 6.36 ppm correlated with the 6-$H_A$ double doublet at δ 6.42 ppm with $J = 8.5$ and 2.5 Hz as well as the 5-$H_A$ at δ 6.65 ppm with $J = 8.5$ and 1.0 Hz. The singlet at δ 6.15 ppm was then assigned to 6-$H_D$, and through NOE experiments that showed enhancement of this signal by irradiation of both the 7-$OMe_D$ and 5-$OMe_D$ at δ 3.85 and 3.82 ppm, respectively, thereby establishing a (4,8) interflavanoid bond.

The methoxyl signal at δ 3.82 ppm was assigned to the 5-$OMe_D$ through NOE to the 4-$H_F$ methylene protons. Similarly, the methoxyl signal at δ 3.72 ppm was assigned to the 7-$OMe_A$ through the enhancement of the 8-$H_A$ doublet at δ 6.36 ppm and to the 6-$H_A$ double doublet at δ 6.42 ppm. The rotational conformation of the interflavanoid bond was then established by the NOE observed between 7-$OMe_D$ at δ 3.85 ppm and 4-$H_C$ doublet at δ 4.83 ppm with $J = 10.0$ Hz. Therefore, the major rotamer is that in which the A(10)—C(4)—D(8)—D(7) dihedral is (−), or the more compact rotamer, in which the E- and F-rings of the lower unit are folded back under the plane of the upper unit A- and C-rings (Fig. 4).

Although rotation about the interflavanoid bond was sufficiently slow at 296–298 K to allow the observation of the two rotamers, interchange did occur, as was evident from NOE association of, e.g., 7-$OMe_D$ to both 4-$H_C$ and 3-$H_C$ when the former resonance of the main rotamer was irradiated. The difference in magnitude of these enhancements, however, permitted the unambiguous assignment of major and minor rotamers. A similar protocol was adopted to deal with the same phenomenon for the derivatives of the remaining compounds.

Notable also is the conspicuous preference for the more crowded conformation (cf. Fig. 4). The effect of adopting such a compressed conformation is presumably to minimize the surface area of the molecule, and hence solute–solvent contact.[15] Consideration of Fig. 4 indicates that this conformation presumably also permits an attracting π–π interaction between the A- and E-rings and between the latter rings and the B-ring, thus stabilizing this apparently non-preferred conformation.[26] Such a preference for the crowded conformation is observed for all the (4,8)-isomers in this study. In the less crowded (+)-rotamer of (1), $J_{2,3F}$ at 6.5 Hz is considerably reduced compared to that of the (−)-rotamer

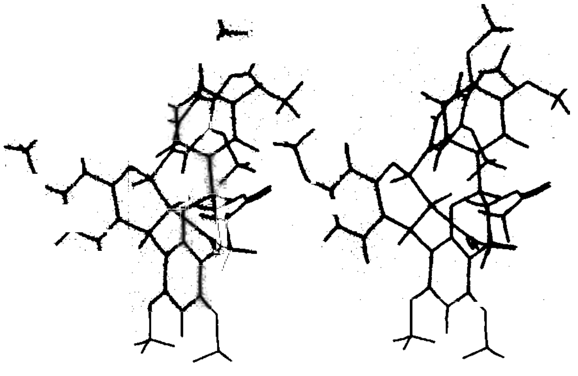


**Figure 4.** Conformation of the major rotamer in fisetinidol-(4α,8)-catechin (1).

(9.0 Hz), thus indicating a more free conformational itinerary for the F-ring which culminates in considerable contributions of A-conformers with concomitant reduction in dihedral angles and coupling constants. The same phenomenon is also operative for the derivative of the (4$\beta$,8)-profisetinidin (2).

Having established the connectivity between the heterocyclic 2-H resonances to the B- and E-ring protons through COSY and NOE experiments, the assignments for the B- and E-rings could be established using the same techniques. The process for assignment of the protons of the B- and E-rings was essentially the same for each compound so only this example will be described in detail. The COSY spectrum showed that the upfield methoxyl at $\delta$ 3.54 ppm was correlated with the *meta*-coupled 2-$H_B$ at $\delta$ 6.68 ppm, $J = 2.0$ Hz (also verified by NOE) and that this proton could also be correlated with the doublet at $\delta$ 6.68 ppm, $J = 8.5$ Hz and the double doublet at $\delta$ 6.73 ppm with $J = 8.5$ and 2.5 Hz assigned to 5-$H_B$ and 6-$H_B$, respectively. The assignment of the 4-$OMe_B$ at $\delta$ 3.81 ppm was confirmed by NOE to the distorted 5-$H_B$ doublet at $\delta$ 6.68 ppm. Similarly, 2-$H_E$, 5-$H_E$ and 6-$H_E$ were assigned through the COSY spectrum that showed correlation between the *meta*-coupled ($J = 2.5$ Hz) doublet at $\delta$ 6.52 ppm with the doublet at $\delta$ 6.64 ppm ($J = 8.5$ Hz) and the double doublet at $\delta$ 6.45 ppm with $J = 8.5$ and 2.5 Hz. NOE between the 2-$H_E$ at $\delta$ 6.52 ppm and the methoxyl at $\delta$ 3.70 ppm and between the doublet at 6.64 ppm and the methoxyl at 3.81 ppm provided confirmation of these assignments.

The conformation of the heterocyclic ring in the upper unit of the minor rotamer was also an approximate half chair (E-conformation) with 2-$H_C$, 3-$H_C$ and 4-$H_C$ appearing at $\delta$ 4.83, $J = 10.0$ Hz; 6.08, $J = 10.0$ Hz; and 4.91 ppm, $J = 10.0$ Hz, respectively. In marked contrast, the lower unit of this rotamer exists in a high proportion (about 50%) of the A-conformer by 2-$H_F$ at $\delta$ 5.06 ppm with $J = 6.5$ Hz.

### 2*R*,3*S*,4*R*-2,3-*trans*-3,4-*trans* Stereochemistry: (4$\alpha$,6) linkage

The $^1$H NMR spectrum of the methyl ether acetate derivative of fisetinidol-(4$\alpha$,6)-catechin (3) in $CDCl_3$ was less complicated showing two rotamers in relative proportions of 2.5:1. The heterocyclic C-ring protons for the two rotamers (Table 1) were assigned as above from a COSY spectrum as summarized. On the basis of the $^3J_{HH}$ coupling in the upper unit heterocyclic C-ring in the two rotamers, the heterocyclic C-ring conformations were the same and predominantly an E-conformation in both rotamers.

The heterocyclic ring protons of the terminal unit F-ring of the major rotamer could then also be assigned (Table 1) from the COSY spectrum as $\delta$ 4.96, $J = 8.0$ Hz; 5.35, m; and 3.14, 16.0 and 5.5 Hz; and 2.78 ppm, 16.0 and 8.0 Hz for 2-$H_F$, 3-$H_F$, 4-$H_{F\alpha}$ and 4-$H_{F\beta}$, respectively. Corresponding assignments for the minor rotamer are $\delta$ 4.84, $J = 9.0$ Hz; 5.27, m; 3.15, $J = 16.0$ and 5.5 Hz; and 2.65 ppm, $J = 16.0$ and 9.0 Hz for 2-$H_F$, 3-$H_F$, 4-$H_{F\alpha}$ and 4-$H_{F\beta}$. The terminal unit $^3J_{HH}$ coupling ($J_{2,3F} = 9.0$ Hz) suggest small (about 10%) proportions of A-conformers, assuming Porter *et al.*'s[19] approach to an arithmetic time-averaging molar ratio of the A- and E-conformers for the observed coupling constants.

Assignment of the rotational conformation of the two rotameric pairs was accomplished through a series of NOE experiments, first to assign the methoxyl signals. Most significant of these were NOE experiments involving the A- and D-ring methoxyls, which importantly also verified the (4,6) linkage due to NOE from only one methoxyl (the 7-$OMe_D$ at $\delta$ 3.53 ppm and its minor rotameric counterpart at $\delta$ 3.77 ppm) to the two 8-$H_D$ singlets at $\delta$ 6.27 and 6.28 ppm that were observed in relative proportions of 2.5:1, respectively. The overlapping A-ring methoxyls at 3.72 ppm were assigned through NOE between the 7-$OMe_A$ and the two 8-$H_A$ doublets ($J = 2.5$ Hz) that appeared at $\delta$ 6.44 and 6.46 ppm in relative proportions of about 2.5:1 and to the pair of 6-$H_A$ double doublets ($J = 9.0$ and 2.5 Hz) at $\delta$ 6.38 and 6.42 ppm. The pair of signals assigned to the 5-$OMe_D$ at $\delta$ 3.75 and 3.01 ppm showed NOE to the 5-$H_A$ double doublets at $\delta$ 6.60 and 6.66 ppm ($J = 8.5$ and 1.0 Hz) with weaker enhancement of the 8-$H_A$. The methoxyl signals downfield from $\delta$ 3.80 ppm all showed NOE to the B- and E-rings.

With the methoxyl signals assigned, the conformation of the major rotamer was clearly defined by the NOE observed from the 7-$OMe_D$ to 3-$H_C$ and the 5-$OMe_D$ to 4-$H_C$ and 5-$H_A$. Therefore, it corresponds to the rotamer in which A(10)—C(4)—D(6)—D(7) is (+) or the pyran oxygen is back behind the plane of the C-4—D-6 interflavanoid bond, which is the more compact rotamer conformation (Fig. 5).

### 2*R*,3*S*,4*R*-2,3-*trans*-3,4-*cis* Stereochemistry: (4$\beta$,8) linkage

The $^1$H NMR spectrum of the methyl ether acetate derivative of the (4$\beta$,8)-linked dimer 2 in $CDCl_3$ showed two rotamers that occurred in relative proportions of about 2:1 (Table 1). A COSY spectrum permitted assignments for the major and minor rotamers as summarized in Table 1. Coupling constants of the hetero-

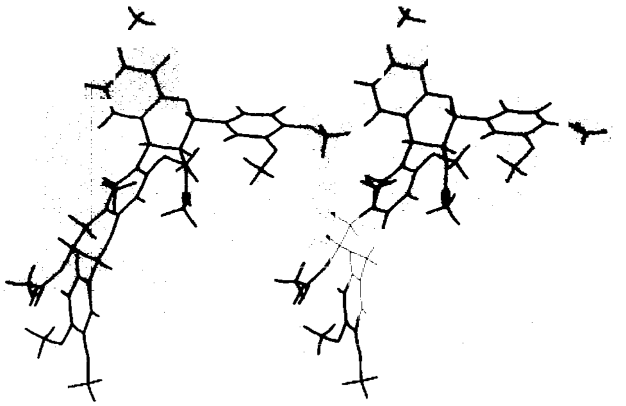


**Figure 5.** Conformation of the major rotamer in the methyl ether acetate derivative of fisetinidol-(4$\alpha$,6)-catechin (3).

cyclic C-ring indicated that the upper unit of both the major and minor rotamers were predominantly of a half-chair E-conformation.

The F-rings in the major and minor rotamers of the derivative of **2** exists as a mixture of A- and E-conformations. Although the E-conformer dominates in the (+) rotamer, large proportions (about 40%) of A-conformer are present in the (−) rotamer, as indicated by the $J_{2,3}$ coupling of 9.0 Hz for the (+) and 7.0 Hz for the (−) rotamer (Table 1). The interrelationship demonstrated between rotational and C-ring conformational properties demonstrated in this compound is especially interesting.

Using the methoxyls of the A- and D-rings, NOE experiments define both the (4,8) interflavanoid bond and the stereochemistry at C-4. All of these methoxyls are doubled because of rotational isomerism. The 7-$OMe_A$ of the major rotamer at $\delta$ 3.46 ppm can be assigned by the NOE observed to the 8-$H_A$ doublet at $\delta$ 5.73 ppm, $J = 2.5$ Hz and to the 6-$H_A$ double doublet at $\delta$ 6.22 ppm with $J = 8.0$ and 2.5 Hz. The 7-$OMe_D$ of the major and minor rotamers appears at $\delta$ 3.79 and 3.33 ppm, respectively, both showing NOE to the 6-$H_D$ singlets at 6.16 and 6.08 ppm (Table 2). Importantly, observation of NOE of the 2-$H_C$ doublet at 5.27 ppm, from irradiation of the 7-$OMe_D$ in the minor rotamer, establishes the 4$\beta$ linkage. The COSY spectrum also showed long-range correlation between 4-$H_C$ and 6-$H_D$. Only 5-$OMe_D$ in the minor rotamer at $\delta$ 3.76 ppm was resolved sufficiently to perform NOE experiments, but this also showed enhancement of the 6-$H_D$ singlets, thus proving the (4,8) interflavanoid bond.

The conformation of the major rotamer is (+), where E- and F-rings of the lower terminal unit are back and above the plane of the A- and C-rings of the upper unit, as shown by the NOE observed from the 7-$OMe_D$ of the major rotamer at $\delta$ 3.79 ppm to the 4-$H_C$ doublet at 4.89 ppm.

## 2*R*,3*S*,4*S*-2,3-*trans*-3,4-*cis* Stereochemistry: (4β,6) linkage

The $^1H$ NMR spectrum of the methyl ether acetate derivative of the (4$\beta$,6)-linked dimer **4** in $CDCl_3$ showed two rotamers in approximately equal proportions (Table 1). Therefore, it was necessary to refer to a COSY spectrum in order to assign the signals belonging to each rotamer. Doublets at $\delta$ 5.22 and 5.28 ppm (with $J = 9.0$ Hz, respectively) were assigned to 2-$H_C$; two double doublets centered at about $\delta$ 5.52 ppm ($J = 9.0$ and 6.5 Hz) and 5.55 ppm ($J = 10.0$ and 6.5 Hz) were assigned to 3-$H_C$; and $\delta$ 4.71 and 5.02 ppm (both with $J = 6.5$ Hz) were assigned (see below) to 4-$H_C$ of the (−) and (+) rotamers, respectively. On the basis of $J_{2,3}$ of 9–10 Hz and $J_{3,4}$ of 6.5 Hz, the 2,3-*trans*-3,4-*cis* stereochemistry with the heterocyclic ring of the upper unit of both rotamers approximating a half-chair E-conformation is evident.

This compound was especially interesting because the conformation of the lower terminal unit differed so dramatically in the two rotamers. The two 2-$H_F$ protons appeared at $\delta$ 4.91 ppm with $J = 9.0$ Hz and at $\delta$ 5.20 ppm with $J = 5.0$ Hz, showing that in the latter (+) rotamer the lower unit existed predominantly in an A-conformation.

As in the previous examples, allocation of the different sets of resonances to the (−) or (+) rotamers was accomplished by NOE experiments, using the A- and D-ring methoxyls, each of which appeared as two signals of approximately equal intensity. The Ar-H protons were first correlated with the two sets of heterocyclic ring protons through a COSY spectrum. This spectrum indicated correlation between the 4-$H_C$ at $\delta$ 4.71 ppm of one rotamer with 8-$H_D$ at $\delta$ 6.30 ppm, 8-$H_A$ at $\delta$ 6.51 ppm, and 5-$H_A$ at 6.77 ppm. Corresponding assignments for the other rotamer were made through correlation of the 4-$H_C$ at $\delta$ 5.02 ppm with the 8-$H_D$ at 6.36 ppm, 8-$H_A$ at $\delta$ 6.51 ppm and 5-$H_A$ at $\delta$ 6.88 ppm. This connects upper to lower units in each rotamer. Unequal populations and thus dissimilar integral intensities observed for rotamers in the previous examples formed an integral part of the strategy to allocate unambiguously different sets of resonances to the major and minor rotamers, respectively. Differentiation of the rotamers via selected NOE enhancements were normally preceded by COSY experiments where $^4J_{HH}$ couplings were extensively utilized to link aromatic protons with the relevant heterocyclic resonances. The absence of $^4J_{HH}$ couplings between the D- and F-rings of the catechin terminal units, however, necessitates the signal intensities to be used in assigning the terminal units but equal proportions of rotamers observed for the (4$\beta$,6)-linked dimer prevent their assignment. Differentiation between the terminal units in the case dimer of

**Table 2. NOE effects typically employed to differentiate between rotational isomers**

| Compound | NOE observed between | Rotamer | NOE (%) |
|---|---|---|---|
| Fisetinidol-(4$\beta$,8)-catechin (**2**) | 7-$OMe_D$ and 6-$H_D$ | Major | 11.9 |
| | 7-$OMe_D$ and 4-$H_C$ | Major | 1.3 |
| | 7-$OMe_D$ and 6-$H_D$ | Minor | 8.1 |
| | 5-$OMe_D$ and 6-$H_D$ | Minor | 12.0 |
| | 7-$OMe_D$ and 2-$H_C$ | Minor | 1.1 |
| *ent*-Fisetinidol-(4$\beta$,8)-catechin (**6**) | 7-$OMe_D$ and 6-$H_D$ | Major | 8.5 |
| | 7-$OMe_D$ and 4-$H_C$ | Major | 1.2 |
| | 7-$OMe_D$ and 6-$H_D$ | Minor | 6.0 |
| | 5-$OMe_D$ and 6-$H_D$ | Minor | 7.6 |

4 is based on the $^5J_{HH}$ coupling observed between 8-H$_D$ and 4-H$_{F\alpha}$ for the (−) rotamer in the COSY spectrum.

Once the Ar-H protons were assigned it was possible to identify the methoxyl signals and, through NOE experiments using the 5- and 7-methoxyls of the D-ring, define the rotational conformation of each. The problem was complicated by superposition of the 7-OMe$_A$ in one rotamer with the 7-OMe$_D$ in the other rotamer at $\delta$ 3.74 ppm. However, assignment of the 7-OMe$_D$ for the (−) rotamer at $\delta$ 3.38 ppm was clear from NOE with the 8-H$_D$ at $\delta$ 6.30 ppm and that for the (+) rotamer by the strong NOE observed at $\delta$ 6.36 ppm together with the H-8$_A$ and H-6$_A$ of the (−) rotamer from irradiation of the methoxyl signal at $\delta$ 3.74 ppm. Assignment of the resonance at $\delta$ 2.69 ppm to the 5-OMe$_D$ of the (+) rotamer (Fig. 6) was then clear from the NOE observed in both the 8-H$_A$ and 2-H$_C$ signals. By contrast, irradiation of the 5-OMe$_D$ of the other rotamer results in enhancement of the 4-H$_C$ and irradiation of the 7-OMe$_D$ results in enhancement of 2-H$_C$, thus confirming this as the (−) rotamer.

### 2*S*,3*R*,4*R*-2,3-*trans*-3,4-*trans* Stereochemistry: (4β,8) linkage

The $^1$H NMR spectrum of the methyl ether acetate of *ent*-fisetinidol-(4β,8)-catechin (**6**) displayed a rotamer ratio of 1:1.4 when recorded in CDCl$_3$. The distinct resonances were assigned primarily through the aid of a COSY spectrum while differentiation between rotational isomers was based on NOE enhancements (Table 2).

The $J_{2,3C}$ couplings (Table 1) indicate that the heterocyclic C-rings in both rotamers closely approximate 'reverse' half-chairs and predominantly E-conformations. The conformation of the F-ring heterocycles in the terminal units were characterized by high proportions of A-conformations with the minor rotamer showing slightly higher proportions of A-conformations than the major rotamer, as was evident from the coupling constants ($J_{2,3F}$ = 6.0 and 7.0 Hz, respectively). These small coupling constants (Table 1) suggest **A**:**E** ratios of about 1:1 for 2,3-*trans* isomers. The tendency of the F-ring to adopt an A-conformation in both rotamers presumably again reflects the aptitude of these molecules to minimize surface area in CDCl$_3$ solutions.

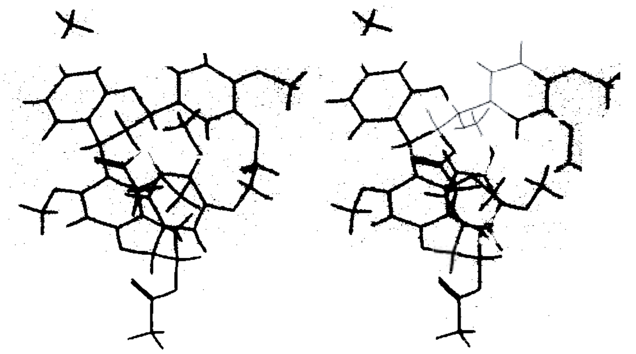


**Figure 6.** Conformation of the (+) rotamer in the methyl ether acetate derivative of fisetinidol-(4β,6)-catechin (**4**) with an A-conformation of the terminal unit.

Although similar proportions of rotamers were observed, differences in signal intensities as reflected in integral values were adequate to allow, in combination with COSY data, the unambiguous assignment of all the resonances for both rotamers. Assignment for 6-H$_D$ of the major and minor rotamer at $\delta$ 6.13 and 6.06 ppm respectively, were verified by NOE experiments.

Although NOE experiments were complicated by rotamer interchange, the difference in magnitude of NOE enhancements facilitates unambiguous assignments. As was observed in previous examples, most methoxyl signals downfield from $\delta$ 3.80 ppm were attributable to substituents on the B- and E-rings again observed here by the strong NOE to the Ar-H of these rings. NOE enhancement between the respective D-ring singlets ($\delta$ 6.13 and 6.06 ppm for the major and minor rotamers) and the methoxyl signals at $\delta$ 3.86 and 3.80 ppm for the major and $\delta$ 3.55 and 3.75 ppm for the minor rotamer, not only differentiate the D-ring methoxyls but also confirm the proposed (4,8) interflavanyl link. Weak but significant NOE enhancement observed for the major rotamer between 4-H$_C$ ($\delta$ 4.81) and D-ring methoxyl at $\delta$ 3.86 clearly claim the latter as 7-OMe$_D$ and the major rotamer as that in which the A(10)—C(4)—D(8)—D(7) dihedral angle is (+) where the lower terminal unit D-ring extends out away and the E-ring extends back to the A- and C-ring plane of the upper extender unit (Fig. 7).

### 2*S*,3*R*,4*S*-2,3-*trans*-3,4-*cis* Stereochemistry: (4α,8) linkage

The $^1$H NMR spectrum of the methyl ether acetate derivative of *ent*-fisetinidol-(4α,8)-catechin (**5**) in CDCl$_3$ showed two rotamers in relative proportions of about 2:1. Even though the major and minor rotamers were readily distinguishable on the basis of signal intensity, a COSY spectrum was helpful in assigning the proton spectrum. This compound represents one of the most interesting of the series owing to the unusually small coupling constants for the C-ring of the minor rotamer. The 2-H$_C$, 3-H$_C$ and 4-H$_C$ appeared at $\delta$ 5.04, $J$ = 10.0 Hz; 5.47, $J$ = 10.0 and 7.0 Hz; and 5.02 ppm, $J$ = 7.0 Hz for the major rotamer and at $\delta$ 5.29, $J$ = 7.5 Hz; 5.64, $J$ = 7.5 and 6.5 Hz; and 4.86 ppm, $J$ = 6.5 Hz for the minor rotamer. The conformation of the hetero-

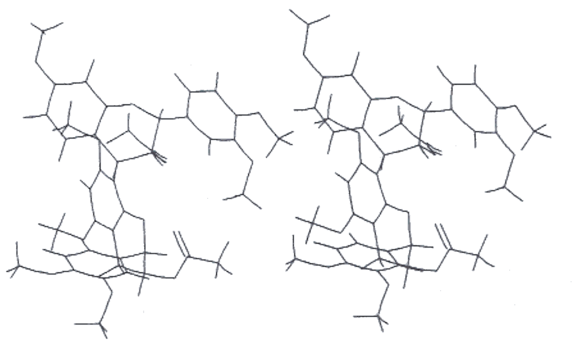
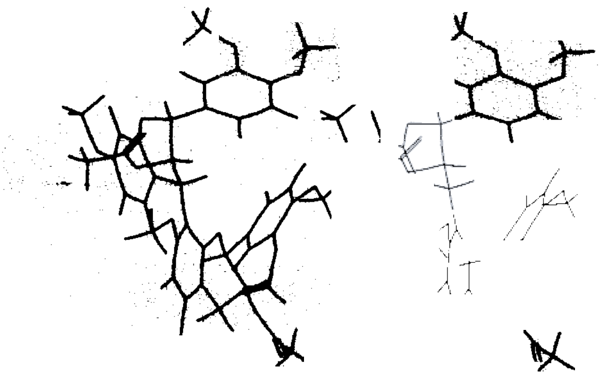


**Figure 7.** Conformation of the minor rotamer of *ent*-fisetinidol-(4β,8)-catechin (**6**) with an A-conformation of the terminal unit.

cyclic C-ring in the major rotamer closely approximated a 'reverse' half-chair E-conformation. Assuming A- and E-flip that would be time averaged on the NMR timescale as suggested by Porter *et al.*[19], and observed in molecular dynamics studies of tetra-*O*-methylcatechin,[9] the minor rotamer existed with substantial proportions (about 30–35%) of the C-ring in an A-conformation (Fig. 8). We cannot explain the absence of a similar phenomenon in the methyl ether acetate derivative of the fisetinidol-(4$\beta$,8)-catechin analogue **2**.

The heterocyclic rings of the terminal unit were of similar conformation in both rotamers with $J_{2,3F}$ approximating 8.0 Hz (Table 1). Therefore, a significant proportion (about 25%) of the time-averaged conformation of the terminal unit was in the A-form.

An NOE experiment showed that the methoxyl signal at $\delta$ 3.64 ppm had to be assigned to the 7-OMe$_A$ by enhancement of the 8-H$_A$ at $\delta$ 6.08 ppm with 2.0 Hz *meta* coupling and to the 6-H$_A$ double doublet at $\delta$ 6.34 ppm. To assign the D-ring methoxyl signals the two 6-H$_D$ singlets, one at $\delta$ 6.14 ppm for the major rotamer and one at 6.10 ppm for the minor rotamer were irradiated. The methoxyl signal at $\delta$ 3.78 and 3.81 ppm that were correlated with the major rotamer 6-H$_D$ proton were then irradiated to show a weak but significant enhancement of the 4-H$_C$ proton from the methoxyl at 3.78 ppm. Therefore, the major conformer was the (−) rotamer.



## CONCLUSIONS

We have demonstrated a remarkable conformational mobility of both the interflavanoid bond and of the C- and F-ring heterocycles of the heptamethyl ether diacetates of profisetinidin biflavanoids representing constituent units of the economically important wattle and quebracho tannins. High-resolution $^1$H NMR experiments have solved long-standing questions concerning the duplication of signals due to rotational isomerism at ambient temperature, and provided evidence needed to define the configurations of both rotamers. The observation that the more compressed conformation is indeed preferred is presumably explicable in terms of the aptitude of these molecules to minimize their effective surface area and hence solute–solvent interactions as hypothesized by Foo and Porter[15] in their consideration of peracetate derivatives. In the analogues that permit considerable freedom in the conformational itinerary of the terminal unit, A-conformers play an important part in the overall time-averaged shape of the terminal units. These results provide a critical foundation from which to build a more complete understanding of the conformational flexibility of natural phenolic analogues.

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