

## Heterogeneous Fluorescence Decay of (4→6)- and (4→8)-Linked Dimers of (+)-Catechin and (-)-Epicatechin as a Result of Rotational Isomerism

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**Abstract:** The time-resolved fluorescence of (+)-catechin and (-)-epicatechin decays as a single exponential. In contrast, dimers formed from (+)-catechin and (-)-epicatechin have more complex decays unless rotation about the interflavan bond is constrained by the introduction of a new ring. The fluorescence decay in unconstrained dimers is adequately described by the sum of two exponentials. In a peracetylated dimer, the relative preexponential factors are in excellent agreement with the relative populations of two rotational isomers deduced from high-resolution NMR spectra. Removal of the acetyl groups does not significantly change the ratio of the preexponential factors, but it yields a first-order NMR spectrum. The reduction in the energy barrier between the rotational isomers upon removal of the acetyl groups causes interconversion of the isomers to become fast on the NMR time scale. However, resolution of the two populations is maintained on the fluorescence time scale.

(+)-Catechin and (-)-epicatechin are the monomer units in a class of polymers, called condensed tannins or polymeric proanthocyanidins, that are found in a wide variety of plants.<sup>1-3</sup> It is believed that these plant polymers act as defense mechanisms for plants.<sup>4,5</sup> They are thought to protect plants from herbivores by forming complexes with salivary proteins, giving rise to a highly astringent taste.<sup>6-8</sup> The polymeric proanthocyanidins also form

indigestible complexes with the plant proteins, thereby limiting the nutritional value of the vegetation.<sup>9</sup> Condensed tannins are

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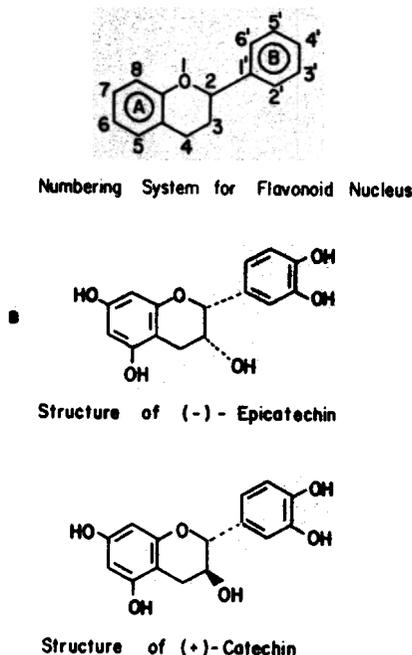


Figure 1. Structure and numbering system for epicatechin and catechin.

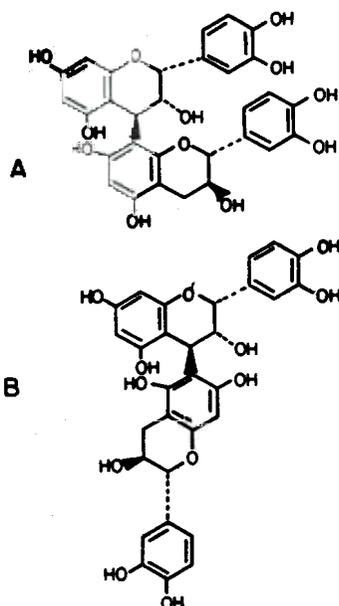


Figure 2. Structures of (A) epicatechin-(4 $\beta$ →8)-catechin (procyanidin B-1) and (B) epicatechin-(4 $\beta$ →6)-catechin (procyanidin B-7).

also credited with a role of protecting plants from microorganisms, the tannins in the seed coats of peanut skins being only one of many such examples.<sup>10</sup>

Polymeric proanthocyanidins with different hydroxylation patterns on the aromatic A and B rings of the basic flavanoid structure (Figure 1) have been found widely distributed in the plant kingdom. The most commonly found proanthocyanidins are those made up of the 3,3',4',5,7-pentahydroxy flavans of either (2*R*,3*R*)-[(-)-epicatechin] or (2*R*,3*S*)-[(+)-catechin] absolute stereochemistry. The polymeric proanthocyanidins are usually formed by linking the monomer units from C(4) to C(8) or from C(4) to C(6).<sup>11-15</sup> The bond that links the two monomer units

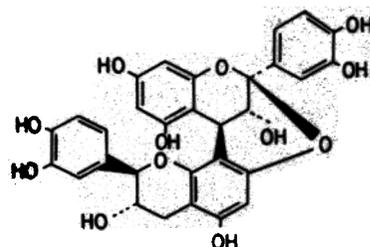


Figure 3. Structure of bridged dimer procyanidin A-1, epicatechin-(4 $\beta$ →8;2 $\beta$ →O→7)-catechin.

is referred to as the interflavan bond. Two dimers that illustrate the different linkage patterns are depicted in Figure 2. The conformations of these dimers are the subject of this investigation.

The <sup>1</sup>H NMR spectra of peracetylated derivatives of proanthocyanidin dimers at ambient temperatures show two rotational isomers for each dimer, indicating restricted rotation about the interflavan bond.<sup>16,17</sup> Although rotational isomerism has been observed by <sup>1</sup>H NMR for acetylated dimers of catechin and epicatechin, the <sup>1</sup>H NMR spectra of the two isomers are not resolved for the free phenol forms of these dimers. Nevertheless, energy minima from molecular mechanics (MM2) calculations on a series of C(4)-C(8) and C(4)-C(6) linked dimers of (+)-catechin and (-)-epicatechin support a twofold rotation about the interflavan bond.<sup>18</sup> The energy difference between the two minima is small enough that both minima may be populated to a significant extent. The extent of rotational isomerism in the free phenol forms has important consequences for the solution conformations of the polymers. This work will show that the relative populations of the rotational isomers for free phenol forms of dimers of (+)-catechin and (-)-epicatechin may be estimated by time-resolved fluorescence spectroscopy. These experimental results are a necessary prerequisite for the construction of realistic rotational isomeric state descriptions of the polymers.<sup>19,20</sup>

#### Experimental Methods and Details

The monomers were purchased from Sigma Chemical Co. Quinine bisulfate, dioxane (HPLC grade), tetramethylsilane (TMS), deuterated dioxane, and deuterated nitrobenzene were purchased from Aldrich Chemical Co. Deionized distilled water was used throughout. A series of dimeric procyanidins and their peracetate derivatives epicatechin-(4 $\beta$ →8)-catechin, epicatechin-(4 $\beta$ →6)-catechin, and epicatechin-(4 $\beta$ →8;2 $\beta$ →O→7)-catechin was obtained either by synthesis or by isolation from plant tissues as described previously.<sup>10,15</sup> The nomenclature is described by Hemingway et al.<sup>14</sup> This series of compounds provides pairs differing in the location of the interflavanoid bond and type of interflavanoid linkage. Figure 3 shows the structure of the bridged dimer. All other chemicals were reagent grade.

Absorption measurements were performed on a Cary 219 spectrophotometer. All steady-state fluorescence measurements were performed at 25 °C on an SLM 8000 spectrofluorometer interfaced to an Apple II+ microcomputer. The fluorescence data were collected as a ratio of sample signal to reference signal. Polarizers were oriented at 0° and 55° to correct for anisotropic effects.<sup>21</sup> Excitation was at 272, 280, and 288 nm (4-nm band-pass), and emission was measured from 285 to 450 nm (4-nm band-pass). After subtraction of background fluorescence from

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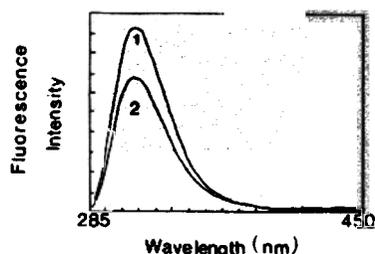


Figure 4. Steady-state fluorescence spectra of (1) epicatechin-(4 $\beta$ →8)-catechin (0.0056 mg/mL in dioxane) and (2) epicatechin-(4 $\beta$ →6)-catechin (0.0053 mg/mL in dioxane). Excitation wavelength is 280 nm.

a solvent blank, the emission spectra were corrected for wavelength-dependent instrument response. The fluorescence quantum yield was obtained by using quinine bisulfate in 1.0 N sulfuric acid as a standard.<sup>22,23</sup>

Single-photon counting<sup>24</sup> was used to determine the lifetimes of proanthocyanidins in solution. Fluorescence lifetimes were measured in a Photochemical Research Associates nanosecond fluorometer equipped with a Tracor Northern TN-1750 multichannel analyzer. The flash lamp was operated at a repetition rate of 25 kHz, with 6.0 kV applied across a 3.5–4.0-mm electrode gap under a D<sub>2</sub> pressure of 70 kPa. The lamp pulse width was 2.4–2.6-ns fwhm. The excitation wavelength was selected through a 10-nm band-pass interference filter with transmission at 280 nm (MicroCoatings, Westford, MA). Since the excitation light is unpolarized, the emission was detected through one polarizer oriented at 35°. Emission wavelengths of 310–335 nm were selected by an Instruments SA H-10 monochromator (16-nm band-pass). Samples were maintained at 25 °C. Data acquisition was controlled by a Digital Equipment Corp. MINC-11 computer.

Fluorescence decay data were collected by alternating between a reference fluorophore and a proanthocyanidin sample. The counting rate was 100–250 Hz, and the sample dwell time was 200 s/cycle. The decay curves were stored in 512 channels of 0.0549 ns/channel. The experiment was continued until the sample decay curve had about 10 000 counts in the peak. The reference fluorophore was *p*-terphenyl in 75% ethanol/25% water containing 0.8 M KI. The lifetime of the quenched terphenyl solution was determined in an independent experiment using *p*-terphenyl in cyclohexane as the monoexponential standard. The decay of the quenched terphenyl gave a good fit to a monoexponential function with lifetime of 0.20 ns. The 0.95-ns lifetime obtained for *p*-terphenyl in cyclohexane agreed with the literature value.<sup>25</sup>

The lifetimes of (+)-catechin and (–)-epicatechin were measured in water and dioxane. The concentrations in water were 0.015 and 0.014 mg/mL, respectively. The concentrations of both monomers in dioxane were 0.005 mg/mL. The lifetimes of epicatechin-(4 $\beta$ →8)-catechin, its decaacetate, epicatechin-(4 $\beta$ →6)-catechin, and epicatechin-(4 $\beta$ →8;2 $\beta$ →O→7)-catechin (Figure 3) were measured in dioxane at concentrations of 0.011, 0.012, 0.011, and 0.006 mg/mL, respectively. At these concentrations the absorbance at 280 nm did not exceed 0.1.

The fluorescence decay data were deconvolved by using the reference decay in a least-squares analysis.<sup>26</sup> The data were fitted assuming a sum of exponentials

$$F(t) = \sum a_i \exp(-t/\tau_i) \quad (1)$$

where  $a_i$  is the preexponential weighting factor and  $\tau_i$  is the fluorescence lifetime of the  $i$ th component. The value of the reference lifetime was fixed in the deconvolution of proanthocyanidin decays. The goodness of fit was determined by the value of reduced  $\chi^2$ , and the quality of the fit was judged by the shape of the autocorrelation function of the weighted residuals.<sup>27</sup> In addition to single-curve analyses, decay curves for a sample at various emission wavelengths were simultaneously deconvolved in a global analysis,<sup>28</sup> assuming that the lifetimes but not the preexponential factors remain constant across the emission spectrum.

The <sup>1</sup>H NMR spectra for epicatechin-(4 $\beta$ →8)-catechin decaacetate in deuteriated dioxane and deuteriated nitrobenzene were obtained on a Bruker AM 400 NMR. The concentration of the dimer was about 5 mg/mL. The spectrum was recorded initially in deuteriated dioxane

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Table I. Fluorescence Decay Data for Monomers and Dimers<sup>a</sup>

compd <sup>b</sup>	solvent	lifetimes, ns		$a_1/(a_1 + a_2)$	$\chi^2$ range
		$\tau_1$	$\tau_2$		
catechin	dioxane	2.00 ± 0.05		1.00	1.1–1.6
catechin	water	0.74 ± 0.01		1.00	1.0–1.2
epicatechin	dioxane	2.04 ± 0.05		1.00	1.2–1.5
epicatechin	water	0.62 ± 0.01		1.00	0.97–1.2
epi(4 $\beta$ →8)cat	dioxane	1.25 ± 0.08	2.0 ± 0.4	0.75 ± 0.15	0.94–1.3
epi(4 $\beta$ →6)cat	dioxane	1.08 ± 0.05	1.9 ± 0.3	0.85 ± 0.08	1.0–1.4
epi(4 $\beta$ →8)cat	dioxane	0.48 ± 0.05	1.4 ± 0.2	0.90 ± 0.05	0.99–1.4
epi(4 $\beta$ →8)cat Ac					
epi(4 $\beta$ →8)cat Br	dioxane	1.06 ± 0.1		1.00	1.1–1.3

<sup>a</sup> Mean values and standard deviations from 5–10 experiments. Temperature = 25 °C, excitation wavelength = 280 nm, emission wavelength = 310–330 nm. <sup>b</sup> Epi(4 $\beta$ →8)cat = epicatechin-(4 $\beta$ →8)-catechin, epi(4 $\beta$ →6)cat = epicatechin-(4 $\beta$ →6)-catechin, epi(4 $\beta$ →8)cat Ac = epicatechin-(4 $\beta$ →8)-catechin decaacetate, and epi(4 $\beta$ →8)cat Br = epicatechin-(4 $\beta$ →8;2 $\beta$ →O→7)-catechin.

using tetramethylsilane (TMS) as an internal reference at 25 °C. The chemical shifts are reported relative to TMS. Spectra in deuteriated nitrobenzene were recorded at three temperatures: 25, 100, and 170 °C.

## Results and Discussion

**Steady-State Fluorescence of Monomers and Dimers.** Steady-state fluorescence measurements were performed for the two monomers, (+)-catechin and (–)-epicatechin, in water and dioxane and for the dimers in dioxane. Excitation into the <sup>1</sup>L<sub>b</sub> absorption band resulted in a broad structureless fluorescence emission spectrum with a maximum between 310 and 321 nm. Figure 4 shows the fluorescence spectra of two dimers, epicatechin-(4 $\beta$ →8)-catechin and epicatechin-(4 $\beta$ →6)-catechin, in dioxane. Excitation at 272, 280, and 292 nm resulted in no shift of the emission band. At these excitation wavelengths, the fluorescence quantum yield was independent of excitation wavelength for a particular solvent.

The fluorescence quantum yield for the monomers is about 0.30 in dioxane and 0.10 in water.<sup>29</sup> The dimers have lower quantum yields, with values in dioxane of 0.07 and 0.05 for epicatechin-(4 $\beta$ →8)-catechin and epicatechin-(4 $\beta$ →6)-catechin, respectively. Peracetylated dimers have still lower quantum yields.

**Monomer Lifetime Measurements.** The time-resolved fluorescence emission of the monomers in dioxane and water was also measured. The decay curves of both monomers, regardless of solvent, were adequately fit by monoexponential functions. The  $\chi^2$  values were 1.1–1.5 in dioxane and 1.0–1.2 in water and the autocorrelation functions fluctuated randomly around zero. The fluorescence lifetime of the monomers is sensitive to the solvent system as expected from the fluorescence quantum yields. Table I shows that the lifetime of the monomers is about 3 times greater in dioxane than in water. The quantum yield  $Q$  is proportional to the lifetime

$$Q = \tau/\tau_0 \quad (2)$$

where  $\tau$  is the observed lifetime and  $\tau_0$  is the radiative lifetime of the fluorophore. Since both quantum yields and observed lifetimes differ by a factor of 3 in dioxane and water, the radiative lifetimes must be the same in the two solvents. The lower quantum yields in water are due to additional nonradiative decay processes.

**Dimer Lifetime Measurements.** The lifetime measurements for epicatechin-(4 $\beta$ →8)-catechin, epicatechin-(4 $\beta$ →6)-catechin, and epicatechin-(4 $\beta$ →8)-catechin decaacetate were performed in dioxane. The results are summarized in Table I. The fluorescence decays for epicatechin-(4 $\beta$ →8)-catechin and epicatechin-(4 $\beta$ →6)-catechin were best fit by biexponential functions.

In Figure 5a the decay of epicatechin-(4 $\beta$ →8)-catechin in dioxane is deconvolved assuming a single exponential. A poor fit is indicated by a high  $\chi^2$  value of 2.5 and nonrandom fluctuations of the autocorrelation function. When these data are fit to a sum of two exponentials (Figure 5b), an improved fit results:  $\chi^2$  drops to 1.1 and the autocorrelation function fluctuates ran-

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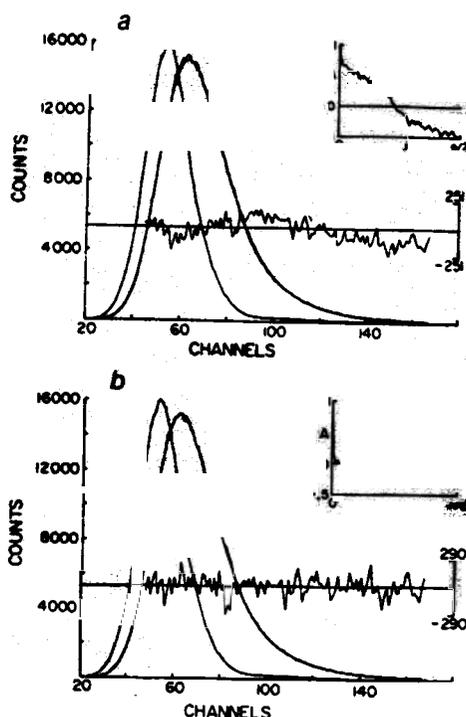


Figure 5. Fluorescence decay curves for epicatechin-(4 $\beta$ →8)-catechin in dioxane. Excitation wavelength is 280 nm. Emission wavelength is 315 nm. Temperature = 25 °C. (a) Data fit to single-exponential function,  $\chi^2 = 2.5$ . (b) Data fit to biexponential function,  $\chi^2 = 1.1$ .

domly about zero. A similar analysis was done for the fluorescence decay of the other free dimer, epicatechin-(4 $\beta$ →6)-catechin, which was also best fit to a biexponential function.

The analysis of the fluorescence decay of epicatechin-(4 $\beta$ →8)-catechin decaacetate in dioxane was more complex. The decaacetate derivative could not be satisfactorily fit by a sum of two exponentials. The poor fit to a biexponential function is indicated by a rather high  $\chi^2$  of 1.9 and nonrandom autocorrelation function. When the data are fit to a sum of three exponentials,  $\chi^2$  falls to 1.2 and a random autocorrelation function results. The third minor component has a lifetime of  $6.8 \pm 0.6$  ns but a preexponential factor that is less than 1% of the sum of the other two preexponential factors. Since it contributes less than 5% of the total fluorescence intensity, we report only the two major components in Table I.

The fluorescence decays of each dimer were measured at several emission wavelengths between 310 and 330 nm. Analysis of the individual decay curves at different wavelengths showed no wavelength-dependent trends in the lifetimes or relative preexponential factors. However, resolution of multiexponential decays from single curves may not be very accurate in cases where the lifetimes differ by less than a factor of 2 or one of the preexponential factors is much larger than the others.<sup>27</sup> Therefore, the decay curves at five emission wavelengths were deconvolved simultaneously in a global program, assuming that the lifetimes are independent of wavelength.<sup>28</sup> The results of the single-curve and global analyses for epicatechin-(4 $\beta$ →6)-catechin in dioxane are compared in Table II. The global  $\chi^2$  of 0.9 indicates an acceptable fit to the model, and the decay parameters from the global analysis are in good agreement with the mean values obtained from single-curve analyses of the data for five emission wavelengths. Thus, it appears that both the lifetimes and preexponential factors are independent of emission wavelength, at least over the range 310–330 nm. The low intensity of the dimer fluorescence precluded measurement at longer wavelength.

The time-dependent fluorescence of the dimers in dioxane departs dramatically from that of the monomers in dioxane. The fluorescence decay for the monomers can be adequately described by a monoexponential function with lifetime of 2.0 ns. However, the fluorescence decay of the dimers in dioxane is heterogeneous. The short-lifetime component (0.5–1.25 ns) has a larger preex-

Table II. Analyses of Fluorescence Decay Curves of Epicatechin-(4 $\beta$ →6)-Catechin in Dioxane at Various Wavelengths<sup>a</sup>

emission wavelength	single-curve analysis				global analysis	
	$a_1/(a_1 + a_2)$	$a_2/(a_1 + a_2)$	$\tau_1$ , ns	$\tau_2$ , ns	$a_1/(a_1 + a_2)$	$a_2/(a_1 + a_2)$
	0.96	0.04	1.16	2.47	0.83	0.17
	0.89	0.11	1.10	1.92	0.83	0.17
	0.77	0.23	1.04	1.70	0.83	0.17
	0.76	0.24	1.01	1.68	0.83	0.17
	0.87	0.13	1.07	1.94	0.82	0.18
av	0.85	0.15	1.08	1.94		
SD	0.08	0.08	0.05	0.29		

<sup>a</sup> Global  $\tau_1 = 1.06$  ns, global  $\tau_2 = 1.79$  ns, global  $\chi^2 = 0.93$ .  $\chi^2$  range for single-curve analysis = 1.0–1.4. Temperature = 25 °C, excitation wavelength = 280 nm.

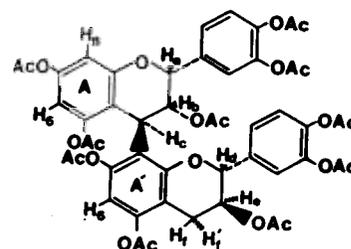


Figure 6. Structure of epicatechin-(4 $\beta$ →8)-catechin decaacetate with protons labeled for NMR assignment.

ponential factor (75–90%) than the longer lifetime component (1.4–2.0 ns).

**Bridged Dimer Lifetime Measurements.** Procyanidin A-1, epicatechin-(4 $\beta$ →8;2 $\beta$ →O→7)-catechin, depicted in Figure 3, has no rotational freedom about its interflavan linkage. The introduction of a new bond between C(2) of the upper unit and the oxygen attached to C(7) of the bottom unit produces a new six-membered ring. This new ring, or bridge, results in a single conformation about the interflavan linkage.

The fluorescence lifetime of the bridged dimer was determined in dioxane. The monoexponential fit with a 1.06-ns lifetime adequately described the fluorescence decay. The  $\chi^2$  values were 1.1–1.3 and the autocorrelation function fluctuated randomly around zero. Furthermore, there was no significant improvement in the fit when a biexponential function was invoked to describe the decay curve. The  $\chi^2$  values were not significantly lower and the autocorrelation function was not improved. This result shows that suppression of the rotation about the interflavan bond in a dimer changes the decay law from a biexponential to a monoexponential function.

**NMR Measurements.** The 400-MHz <sup>1</sup>H NMR spectra of epicatechin-(4 $\beta$ →8)-catechin decaacetate was measured at 25 °C in nitrobenzene. The assignments were made on the basis of chemical shifts relative to TMS, splitting patterns, and direct comparison to the spectra reported by Rauwald<sup>30</sup> for this compound in nitrobenzene. The spectrum we obtained reproduced previous work. According to Rauwald, nine of the protons have dual resonances. The labeling scheme for the assigned protons is given in Figure 6. The assignment of two resonances to one proton can be explained by the existence of two distinct environments that interconvert slowly on the NMR time scale. Rotation about the interflavan linkage, C(4)–C(8), places a particular proton in a different environment. Measurement of the peak integrals yields the relative population of each isomer. Since the H<sub>6</sub> and H<sub>6'</sub> peaks overlap for the minor rotamer, the peak integral for H<sub>6</sub> of the minor rotamer has contributions from both H<sub>6</sub> and H<sub>6'</sub>. The rotamer populations for these two protons were calculated by using eq 3 and 4, where ROT is the major rotamer

$$\text{ROT} = [\text{PI}(\text{H}_6) + \text{PI}(\text{H}_6')]/[\text{PI}(\text{H}_6) + \text{PI}(\text{H}_6') + \text{PI}(\text{H}_6)] \quad (3)$$

$$\text{MROT} = [\text{PI}(\text{H}_6)]/[\text{PI}(\text{H}_6) + \text{PI}(\text{H}_6') + \text{PI}(\text{H}_6)] \quad (4)$$

**Table III.** Comparison of Average NMR Values with Average Preexponential Values for Epicatechin-(4 $\beta$ -8)-Catechin and Epicatechin-(4 $\beta$ -8)-Catechin Decaacetate in Dioxane and Nitrobenzene<sup>a</sup>

dimer	solvent	av NMR data		fluores data $a_1/(a_1 + a_2)$
		maj rot	min rot	
epi(4 $\beta$ -8)cat Ac	NO <sub>2</sub> Benz	0.85	0.15	
epi(4 $\beta$ -8)cat Ac	NO <sub>2</sub> Benz	0.83	0.17 <sup>b</sup>	
epi(4 $\beta$ -8)cat Ac	dioxane	0.84	0.16	0.90 $\pm$ 0.05
epi(4 $\beta$ -8)cat	dioxane	<i>c</i>	<i>c</i>	0.75 $\pm$ 0.15

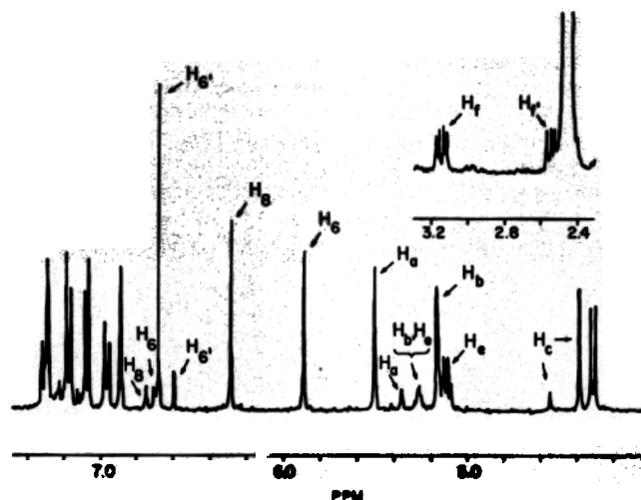
<sup>a</sup> Temperature = 25 °C. NO<sub>2</sub>Benz = nitrobenzene, epi(4 $\beta$ -8)cat Ac = epicatechin-(4 $\beta$ -8)-catechin decaacetate, and epi(4 $\beta$ -8)cat = epicatechin-(4 $\beta$ -8)-catechin. <sup>b</sup> NMR data from Rauwald.<sup>30</sup> <sup>c</sup> First-order NMR spectra reported by Foo and Porter at 25 °C.<sup>17</sup>

population for H<sub>6</sub> and H<sub>6'</sub>, MROT is the minor rotamer population for H<sub>6</sub> and H<sub>6'</sub>, PI is the peak integral for the major rotamer, and PIM is the peak integral for the minor rotamer. The mean values of the rotamer populations from the peak integrals of the nine protons together with the results of Rauwald<sup>30</sup> are given in Table III. Foo and Porter<sup>17</sup> also report rotational isomers by NMR techniques for other decaacetate derivatives of procyanidin dimers in CDCl<sub>3</sub> and nitrobenzene-*d*<sub>5</sub> solvents.

Further evidence for rotational isomerism in epicatechin-(4 $\beta$ -8)-catechin decaacetate was obtained from the <sup>1</sup>H NMR spectra in nitrobenzene-*d*<sub>5</sub> at elevated temperatures. At 25 °C our NMR spectrum showed a major and minor resolved resonance for each of the diagnostic protons. At 100 °C these peaks were broadened and the major and minor resonances were no longer distinguishable. Finally, at 170 °C the peaks resharpended and there were no minor peaks. Rauwald<sup>30</sup> reported a similar disappearance of the minor resonances for epicatechin-(4 $\beta$ -8)-catechin decaacetate in nitrobenzene-*d*<sub>5</sub> at 170 °C. Porter and co-workers<sup>16,17</sup> reported first-order <sup>1</sup>H NMR spectra for other acetylated derivatives of (+)-catechin and (-)-epicatechin dimers in nitrobenzene-*d*<sub>5</sub> at elevated temperatures. These previous investigations<sup>16,17,30</sup> all attribute the appearance of major and minor resonances in the dimers at lower temperature to rotational isomerism. At elevated temperatures interconversion of rotational isomers occurs more rapidly and a first-order <sup>1</sup>H NMR spectrum results.

The fluorescence of (-)-epicatechin and (+)-catechin cannot be studied in nitrobenzene. In order to provide a direct comparison of NMR and fluorescence measurements, the <sup>1</sup>H NMR spectrum of epicatechin-(4 $\beta$ -8)-catechin decaacetate in dioxane-*d*<sub>6</sub> was measured at room temperature. The spectrum in Figure 7 shows dual resonances, which were assigned to a single proton as in the case of epicatechin-(4 $\beta$ -8)-catechin decaacetate in nitrobenzene-*d*<sub>5</sub>. The assignments were based on chemical shifts, multiplicity of peaks, and comparison to published <sup>1</sup>H NMR spectra of similar decaacetate derivatives.<sup>16,17,30</sup> The H<sub>f</sub> and H<sub>f'</sub> peaks for the minor rotamer were not resolved; they may be obscured by some other peaks. The appearance of major and minor peaks for a particular proton indicates that two rotational isomers exist in dioxane. The relative populations of the major and minor rotamer were determined from their peak integrals. The H<sub>b</sub> and H<sub>e</sub> resonances for the minor rotamer could not be separated, since they both occurred at chemical shifts of about 5.27 ppm. Therefore, the peak integral at 5.2 ppm reflects contributions from both H<sub>b</sub> and H<sub>e</sub> of the minor rotamer. The rotamer populations for these two protons were calculated from equations analogous to eq 3 and 4. The relative populations of the major and minor rotamers given in Table III are nearly identical with those obtained for epicatechin-(4 $\beta$ -8)-catechin decaacetate in nitrobenzene.

**NMR and Fluorescence Results for Epicatechin-(4 $\beta$ -8)-Catechin.** The results from <sup>1</sup>H NMR and time-resolved fluorescence for epicatechin-(4 $\beta$ -8)-catechin decaacetate at 25 °C are compared in Table III. The relative magnitudes of the rotamer populations determined by <sup>1</sup>H NMR are similar to those of the preexponential factors for the two lifetime components. These data for the acetylated dimers, taken in conjunction with



**Figure 7.** 400-MHz <sup>1</sup>H NMR spectrum of epicatechin-(4 $\beta$ -8)-catechin decaacetate in dioxane-*d*<sub>6</sub> at 25 °C.

the monoexponential decay for the bridged dimer, suggest that the two lifetime components in the free phenolic dimers arise from two rotational isomers. If it is correct that rotational isomerism in dimers of catechin and epicatechin is responsible for the heterogeneity in the fluorescence decay, then the preexponential factors should reflect the relative populations of the major and minor rotamers.

The preexponential factors in eq 1 depend on a number of variables, including the molar extinction spectrum, the fluorescence emission spectrum, the radiative lifetime, and the concentration of the different fluorescent species. The relative concentrations can be deduced directly from the preexponential factors only if the other variables happen to be the same for all species in the system of interest. The preexponential factors of the dimers are independent of emission wavelength, which implies that the emission spectra of the two rotamers are the same. The fact that the bridged dimer epicatechin-(4 $\beta$ -8;2 $\beta$ -O-7)-catechin and the unconstrained dimers epicatechin-(4 $\beta$ -8)-catechin and epicatechin-(4 $\beta$ -6)-catechin have essentially identical emission spectra supports this notion. The absorption spectral data for these three dimers suggest that the extinction coefficients at 280 nm and the radiative lifetimes of the rotamers are similar. Then under the assumption that the preexponential factors depend mainly on the relative concentrations of the major and minor rotamers, both NMR and fluorescence predict similar populations.

Fletcher et al.<sup>16</sup> report that the free phenolic form of epicatechin-(4 $\beta$ -8)-catechin has a first-order spectrum in deuterated acetone at 25 °C. Foo and Porter<sup>17</sup> also report the absence of major and minor resonances for particular protons in phenolic dimers of (-)-epicatechin and (+)-catechin. However, the fluorescence decay data give evidence of similar rotational populations in the phenolic form of epicatechin-(4 $\beta$ -8)-catechin and the decaacetate derivative. These two observations can be reconciled by consideration of the different time domains for NMR and fluorescence. The difference in the <sup>1</sup>H NMR spectra for the phenolic and decaacetate forms of the dimers at room temperature may be attributed to the presence of bulky acetate groups which increase the rotational barrier in the acetylated dimers. Fletcher's work<sup>16</sup> indicates that in phenolic dimers the rotational barrier is low enough at room temperature so that interconversion is "fast" on the NMR time scale and a first-order spectrum results. Fletcher only observes rotamer populations in the free phenolic dimers in deuterated acetone by <sup>1</sup>H NMR when the temperature is lowered to 0 °C.<sup>16</sup> This observation establishes that rotational isomers are present in the phenolic forms of the dimers and are observable by NMR at low temperatures when the interconversion is slow. Presumably, the nanosecond time scale of fluorescence allows discrimination of the rotational isomers at room temperature. Thus in Table III the preexponential factors of the acetylated and free phenolic forms of epicatechin-(4 $\beta$ -8)-catechin

in dioxane predict similar rotational populations.

Fluorescence decay measurements provide a reasonable estimate of the relative rotamer populations because other variables contributing to the preexponential factors are unaffected by rotational isomerism in this system. This information can be used to construct realistic models that may predict the dimensions and characteristics of (+)-catechin and (-)-epicatechin polymers in solution. It is clear that the existence of rotational isomerism in the dimers has profound implications for the conformations of

the high polymers. This point will receive amplification elsewhere.<sup>19,20</sup>

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**Registry No.** epi(4 $\beta$ →8)cat, 20315-25-7; epi(4 $\beta$ →6)cat, 12798-59-3; epi(4 $\beta$ →8)cat Ac, 21179-20-4; epi(4 $\beta$ →8)cat Br, 103883-03-0; (+)-catechin, 154-23-4; (-)-epicatechin, 490-46-0.