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Temperature Dependence of (+)-Catechin Pyran Ring Proton Coupling Constants as Measured by NMR and Modeled Using GMMX Search Methodology

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Abstract — The pyran ring proton coupling constants for (+)-catechin have been experimentally determined in deuterated methanol over a temperature range of 213 K to 313 K. The experimental coupling constants were simulated to 0.04 Hz on the average at a 90% confidence limit using a LAOCOON method. The temperature dependence of the coupling constants was reproduced from the Boltzmann distribution of the conformational ensemble generated by the GMMX searching program. © 1997, Elsevier Science Ltd. All rights reserved.

Proanthocyanidins (condensed tannins) are an important class of polyphenols found in plant materials. With a growing interest in their interaction with proteins and carbohydrates, understanding their conformational preferences and flexibility has been an important recent goal of researchers.[1,2] The (+)-catechin molecule is representative of the monomers found in many of these polyphenols. The current view of the pyran ring (called the C-ring) conformation in compounds like these is one of a conformer ensemble with structures distributed with the catechol ring on the 2-position (called the B-ring) being in a pseudoequatorial (**E**-conformer) or a pseudoaxial (**A**-conformer) position--see Figure 1. This conformational variation for flavans was first proposed by Fronczek and Mattice[3] based on a pseudoaxial conformation found in the crystal structure of penta-O-acetyl-(+)-catechin.

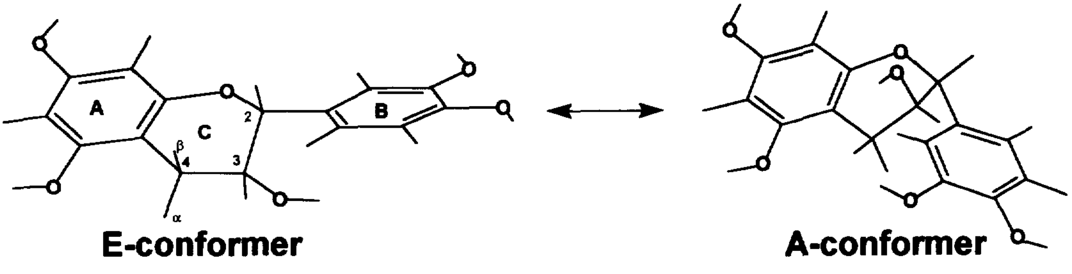

Figure 1. The illustration of the **E**- and **A**-conformers and pyran ring numbering for (+)-catechin.

Porter[4] viewed the $^3J_{HH}$ proton coupling constants as an average between distinct **E**- and **A**-conformers where the energies were determined by MM2 molecular mechanics. Later Mattice[5] examined the **E**- to **A**-conformer transition in tetra-O-methyl-(+)-catechin with molecular dynamics utilizing the Sybyl Tripos force field, starting with the X-ray crystal structure. Tobiason[6] used the GMMX conformational searching program in a mixed systematic/statistical mode with Boltzmann averaging over all conformers to successfully predict simultaneously all three coupling constants in the pyran ring for tetra-O-methyl-(+)-catechin. In a recent NMR study[7] this GMMX technique was further applied to a selection of flavan-3-ol derivatives including (+)-catechin. Although the Boltzmann averaged physical parameters generated from these conformer ensembles look promising, outside of the magnitude of the NMR coupling constants, no other test has been completed on the validity of the GMMX search ensemble as applied to flavanoid compounds.

One important method that allows a test of a molecular ensemble is to examine the temperature dependence of physical parameters predicted from the Boltzmann distribution properties of the ensemble.[6,8,9] Although there has been a wide variety of molecular ensemble computations with dynamics, stochastic and Monte Carlo methods, there have been few temperature studies generated from any current modeling technique to verify the nature of the energy distribution of the conformers in a final selected conformer ensemble.

This paper explores the prediction for the temperature dependence of the $^3J_{HH}$ pyran ring coupling constants in (+)-catechin using the GMMX conformational searching program[10] and the MMX force field compared to that found experimentally by NMR. The purpose is to test the fundamental nature of the molecular ensemble generated by the GMMX protocol.

The initiating structures utilized in the GMMX program to search conformational space were generated in PCMODEL.[11] In all cases, the MMX force field was applied. The conformational search was conducted using the mixed combination Monte Carlo coordinate movements and bond rotations method as was described in the tetra-O-methyl-(+)-catechin study.[6] All degrees of conformational freedom were allowed including hydroxyl group and B-ring rotation. The GMMX program was modified so that the ensemble Boltzmann conformer populations could be expressed at different absolute temperatures. This enabled Boltzmann sums over the ensemble to be computed for a range of temperatures. The basic equations have been described.[6,8] The aromatic carbons were maintained as MMX type-40 atoms since studies on these compounds indicated that running the pi-calculation made little difference in the final coupling parameters. The dielectric constant was set at 5.0, dipole/dipole interactions were used and the hydrogen bond function was turned off.

The experimental NMR chemical shifts and coupling constants (expressed in Hz and recorded from a Varian Unity 300 MHz spectrometer with a 5 mm variable temperature probe) are shown in Table 1 for the range of temperatures from 213 K to 313 K. The reported chemical shifts and proton coupling constants were analyzed from the approximate chemical shifts, coupling constants, line frequencies and intensities obtained from the spectra utilizing the MLDC8 program based on the modified LAOCOON method QCPE #100[12,13] obtained from the Quantum Chemistry Program Exchange. This analysis was supported with the NMR simulation program PCPMR from Serena Software.[14] The chemical shifts were refined to a 90% confidence level of 0.03 Hz on the average, and the coupling constants to within an average value of 0.04 Hz. The MLDC8 program was found to work best in this case using the initial integral transform followed by the automatic top fitting option. The results are further illustrated in the plot of J(Hz) versus temperature (K) shown in Figure 2. The values for these slopes are given in Hz/K.

The coupling constants calculated from the GMMX ensemble, previously reported[7] at room temperature, are Boltzmann averaged using the Altona[15] extension of the Karplus equation and are shown in Figure 3. The calculated and experimental slopes given in Figures 2 and 3 are computed at the 95% confidence limits. This level of agreement between the calculated temperature dependence and the experimental pyran ring proton coupling constants gives added assurance that the ensemble contains many conformers that are representative of the solution mixture. If the low temperature data point at 213 K

Table 1. The temperature dependence of the pyran ring chemical shifts and proton coupling constants in Hz for a 3% solution of (+)-catechin in d_4-methanol.

			Temperature, K			
Par.	213	233	253	273	293	313
w_2	1345.10 (0.02)[a]	1350.24 (0.04)	1356.77 (0.01)	1362.82 (0.02)	1368.43 (0.01)	1373.50 (0.02)
w_3	1179.15 (0.03)	1180.53 (0.06)	1184.32 (0.02)	1187.90 (0.04)	1191.56 (0.04)	1195.30 (0.02)
$w_{4\alpha}$	848.72 (0.03)	847.53 (0.05)	848.51 (0.01)	850.49 (0.02)	853.14 (0.02)	856.12 (0.02)
$w_{4\beta}$	732.62 (0.03)	736.30 (0.05)	741.39 (0.01)	746.23 (0.02)	750.91 (0.02)	755.37 (0.02)
$J_{2,3}$ [b]	7.95 (0.04)	7.69 (0.06)	7.57 (0.02)	7.46 (0.03)	7.44 (0.02)	7.33 (0.02)
$J_{3,4\alpha}$	5.26 (0.05)	5.44 (0.07)	5.43 (0.03)	5.37 (0.04)	5.32 (0.03)	5.32 (0.03)
$J_{3,4\beta}$	8.77 (0.05)	8.53 (0.07)	8.33 (0.02)	8.24 (0.04)	8.11 (0.03)	7.99 (0.03)
$J_{4\alpha,4\beta}$	15.95 (0.04)	15.97 (0.06)	16.11 (0.02)	16.11 (0.03)	16.19 (0.03)	16.17 (0.03)

a) The standard deviations of the simulated NMR spectra in parentheses are computed at the 90% confidence limits.

b) On the automatic (A-mode) of the fitting program, $J_{2,4\alpha}$ and $J_{2,4\beta}$ have coupling constants of - 0.003 and -0.004 Hz, respectively.

is dropped from the data set the slopes become -0.0065 Hz/K, -0.0045 Hz/K and -0.0022 Hz/K, respectively, for the coupling constants $J_{3,4\beta}$, $J_{2,3}$ and $J_{3,4\alpha}$. The slope for $J_{3,4\alpha}$ given as -0.0018 Hz/K is data weighted. It is not well defined with the regression r^2 coefficients ranging from 0.1 to 0.3. The r^2 correlation values on the other two regression lines are 0.97. In an attempt to explain the difference between the magnitude of calculated and experimental $J_{3,4\beta}$ coupling constants, the electronegativity parameter of the Altona equation for pyran carbon-4 proton was examined, but no improvement in values

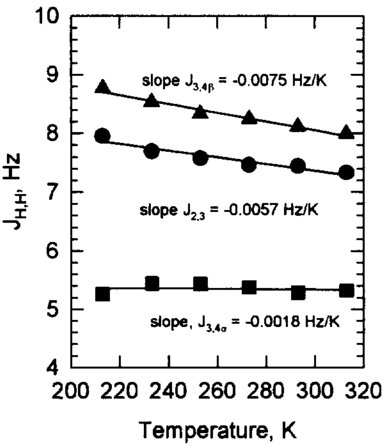

Figure 2. The experimental slopes for the pyran ring coupling constants versus temperature.

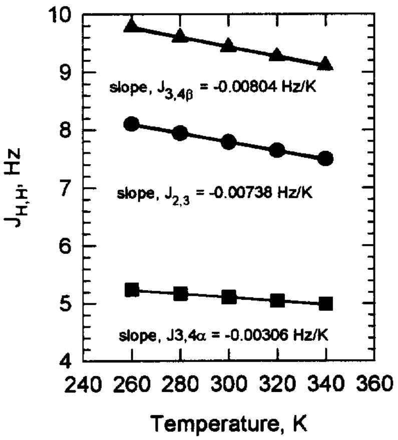

Figure 3. The temperature dependence of the GMMX calculated pyran ring coupling constants.

was established. In addition, the Gibbs free energies were calculated for a series of the lower energy conformers from the GMMX search ensemble using MM3(94) (Tripos, Inc.) with full matrix diagonalization to insure that there was no major reordering of the free energy levels. This was borne out even though MM3 results show a much larger energy difference between the minimum energy **E**-conformer and the lowest energy **A**-conformer.

In conclusion, the satisfactory agreement between calculated and experimental temperature dependence of the pyran ring proton coupling constants in (+)-catechin indicates that the conformer ensemble generated by GMMX has a reasonable energy distribution and contains groupings of conformers that exist in solution.

Experimental—The NMR results were obtained on a Varian Unity instrument operating at 300 MHz using a 5 mm variable temperature probe. The samples were run in d_4-methanol at 1% and 3% concentrations with samples obtained from the Southern Research Station. The temperature controller was calibrated using differences in the chemical shift of methanol and ethylene glycol and had a stability of at least ± 1 K. The samples were run in random order and the temperature was allowed to equilibrate for 15 minutes. The spectra were collected at a spectral width of 4000 Hz, a 60° pulse, 64 k data points with a five-second delay time. Shoulder peak frequencies not obtained from frequency interpolation were obtained from peak peaking on expanded spectra (this was important in the overlap of the C(3)H multiplet). Peak intensities were taken from peak heights.

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