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A Complete Structural and Conformational Investigation of Procyanidin A2 Dimer

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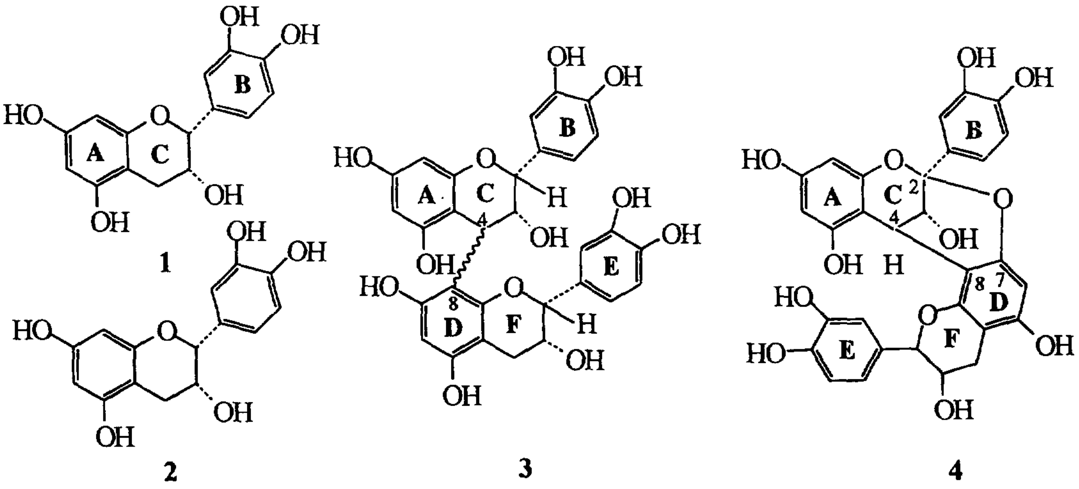
Abstract: Structural and conformational study of A2 procyanidin is presented. By using NMR and molecular mechanics. A demonstration of the C4-C8 bond and of an additional C2-O-C7 ether linkage, characteristics of the procyanidin of A-series, was achieved.

Proanthocyanidins represent one of the major groups of plant polyphenols[1], in various proportion, in different vegetative tissues[2,3]. The structural units are flavan-3-ols : (+)-catechin **1** and (-)-epicatechin **2**. The most common and well known class of dimer procyanidins is the B-series corresponding to a dimer linked in C4-C6 or C4-C8 position[4]. The second class, less studied, is the A-series, wich corresponds to a dimer linked in the C4-C8 position with an additional C2-O-C7 ether linkage[5,6]. Procyanidin B2 **3** was chosen as a model on account the similarity of its structural units (epicatechin-(4β - 8)-epicatechin) with A2 **4** (scheme 1).



Scheme 1

A2 was isolated from the acetone-water extract (7:3, v/v) of horse chestnut seed shells (*Aesculus hyppocastanum*), after extraction with ethyl acetate, by combined LH-20 chromatography and TLC on Silicagel 60 plates. **4** represent 12% of acetone-water dry extract with 84% of purity , measured by reverse-phase HPLC. Mw of 576 (M-H)$^-$ was measued by LSISM^9. The sample was dissolved in methanol-d3. ^{1}H (400.13 MHz) and ^{13}C (100.61 MHz) one- and two- dimensional NMR spectra were recorded in a 5 mm tube on a Bruker DPX 400™ spectrometer using an inverse broad-band probe and gradient field. The structural analysis was performed using the following 2D NMR sequences : COSY, TOCSY, HMBC, HMQC and NOESY.

The integration of protons in the ^{1}H NMR spectrum shows the presence of 15 protons, all magnetically non equivalent (Table 1) (excluding protons of hydroxyl groups). This suggests that a double linkage between ring C and D was present. On the other hand, the ^{1}H-^{1}H TOCSY spectrum indicates that the C ring has lost one proton at carbon C2, since such an experiment, when the mixing time is adequately chosen (in our experiment mt = 40 ms), gives access to the entire spin system. Indeed, we clearly see a correlation between H-C3 and H-C4 alongwith a correlation between H-F2, H-F3 and the two protons of H-F4. Although the HMQC experiment permits the assignment of all the procyanidin carbons directly linked to one or two proton(s), the HMBC experiment gives access, to the interflavonoid linkage types^7 because the visible correlations of HMBC experiments correspond to long range couplings between a given proton and a given carbon over two or three bonds. Figure 1 shows a part of the contour plot of the HMBC spectrum of **4** illustrating the linkage C4-C8 and the linkage C2-O-C7 since the proton linked to C4 (H-C4) correlates with the two carbons D7 and F8a but not with the carbon D5, and the proton linked to D6 correlates with the carbon D5. All the ^{13}C chemical shifts of **4** are reported in table 2 (J measured).

Table 1- ^{1}H and ^{13}C NMR spectral data for **4** .

Carbon number	Multiplicity	Chemical shift (∂ ppm)		Carbon number	Multiplicity	Chemical shift (∂ ppm)		Carbon number	Multiplicity	Chemical shift (∂ ppm)	
		1H	13C			1H	13C			1H	13C
Ring A				**Ring C**				**Ring E**			
4a	C§	-	103.24	2	C	-	99.16	1'	C	-	130.20
5	C	-	156.02	3	C-H	4.07	66.00	2'	C-H	7.18	114.92
6	C-H	6.02	97.28	4	C-H	4.43	28.26	3'	C	-	145.00
7	C	-	157.14					4'	C	-	144.66
8	C-H	6.08	95.47					5'	C-H	6.83	115.00
8a	C	-	153.26					6'	C-H	7.00	118.76
Ring B				**Ring D**				**Ring F**			
1'	C	-	131.45	4a	C	-	101.40	2	C-H	5.95	80.77
2'	C-H	7.15	114.60	5	C	-	151.14	3	C-H	4.26	67.09
3'	C	-	145.30	6	C-H	6.11	95.60	4	C-H2	2.78 / 2.97	28.91
4'	C	-	145.77	7	C	-	155.62				
5'	C-H	6.82	114.65	8	C	-	106.20				
6'	C-H	7.04	118.76	8a	C	-	151.31				

§ Quaternary Carbon.

An attempt was made to propose a 3D structure of **4** . For that purpose we used a previous conformational study performed on procyanidin B2 (**3**)^8. Starting from the conformational file, we chose the conformer with the shortest O (of D)-C2 distance ; then cyclisation was performed. This minimized conformer was subjected to a Monte Carlo conformational study, using MM2* and MM3* force fields, as previously described^9. 3000 steps were run within a 15 kJ.mole^{-1} energy, range resulting in 118 and 110 conformers (respectively from MM2* and MM3*). A cluster analysis conducted with Xcluster 1.1 leads to the same two families considering the torsional angles corresponding to the coupling constants measured by ^{1}H NMR (figure 2). The only difference is that the equatorial family is more stable than the axial one with MM2* (ΔE = 5.6 kJ.mole^{-1}) ; the reverse is true with MM3* (ΔE = 2.4 kJ.mole^{-1}). The coupling constants measured clearly correspond to an equatorial conformer. MM2* led to the best energetic analysis (equatorial more stable than axial), but MM3* gives the best torsion angles (Table 2). So we chose the equatorial conformer obtained with MM3* as the best representation of the A2 molecule (Figure 3). The F ring was found in a half chair conformation (data not shown).

Table 2- Coupling constants measured (^{1}H NMR, 400 MHz) and calculated (MM2* and MM3* force field).

Coupling constance (J)	**Values (Hz)**				
	Measured	**MM2***		**MM3***	
		eq	ax	ax	eq
4J A6-A8	2.2				
4J B2'-B6'	2.2				
3J B5'-B6'	8.3				
3J C2-C3	3.5	4.4	4.5	3.4	3.4
3J E2'-E6'	1.9				
3J E5'-E6'	8.1				
3J F2-F3	< 1.5	0.4	4.4	3.9	0.7
3J F3-F4	2.2	2.9	10.7	10.9	2.1
3J F3-F4'	4.8	3.1	5.7	5.3	4.3
2J F4-F4'	17.1				

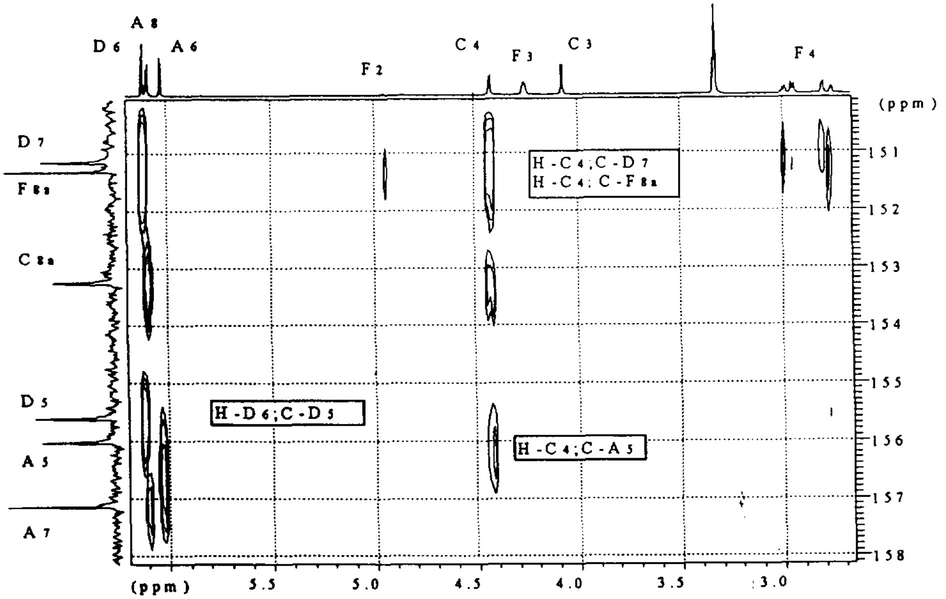


Figure 1- Part of the HMBC contour plot spectrum of **4**. Only correlations permitting the caracterisation of interflavonoid linkage are indicated.

The NOESY experiment on **4** shows few correlations : H-B2' and H-B6', on one hand, and H-E6' and H-B6', on the other hand, exhibit dipolar coupling with H-C3 and H-F3 respectively. These results are in accordance with the modelling since the distance measured between H-C3 and H-B2' / H-B6' (2.77, 4.32 Å, respectively) and H-F3 and H-E2' / H-E6' (3.57 ; 3.68 Å, respectively) are small enougth to observe direct spin-spin interaction. Correlation are also observable between H-C3 and H-C4 (2.50 Å) and between H-F3 and H-F4 / H-F4' (2.38 ; 2.59 Å, respectively), confirming the calculated structure. Contrary to the observation of Foo[10] done upon type A procyanidins, no dipolar coupling was evidence between H-C4 and H-A6 or H-D6 ; nevertheless, such a result is in accordance with the calculated structure since those distances are too large (> 4.50 Å) in order to see such correlations (Figure 3).

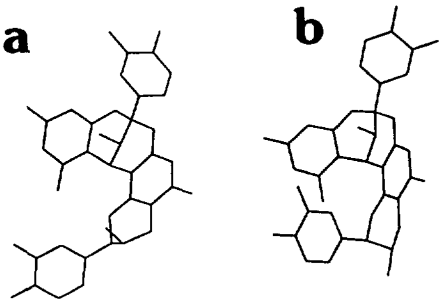


Figure 2- Representation of the two leaders of the cluster analysis (a) equatorial conformation and (b) axial conformation.

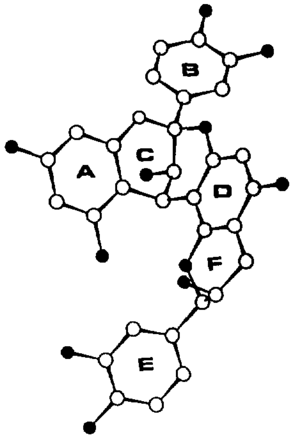


Figure 3- Spatial structure of A2 procyanidin.

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