Structure Elucidation of Procyanidin Oligomers by Low Temperature ^{1}H NMR Spectroscopy

Tuba Esatbeyoglu[1], Beate Jaschok-Kentner[2], Victor Wray[2], and Peter Winterhalter[1*]

[1] *Institute of Food Chemistry, Technische Universität Braunschweig, Schleinitzstraße 20, 38106 Braunschweig, Germany*

[2] *Helmholtz Centre for Infection Research, Inhoffenstraße 7, 38124 Braunschweig, Germany*

TITLE RUNNING HEADER: ^{1}H NMR Spectroscopic Studies of Procyanidins

*Author to whom correspondence should be addressed.

Telephone: +49-531-391-7200; fax: +49-531-391-7230; e-mail: p.winterhalter@tu-bs.de

Supporting Information Available

Phloroglucinolysis data: Confirmation of the structures of the procyanidins **1**-**10** was furnished by acid-catalyzed degradation (phloroglucinolysis). The cleavage of the interflavanoid bonds releases the extension units as carbocations, which reacted with the nucleophilic reagent (phloroglucinol) to form stable flavan-3-ol-phloroglucinol adducts (*28*). The terminal units are obtained as the corresponding flavan-3-ols. These cleavage products were then identified by reversed phase HPLC-PDA and ESI/MS analysis.

Complete degradation revealed that **1** consists of extension units of two equivalents of (–)-EC-ph and a terminal unit of one equivalent of (–)-EC. The nature of the interflavanoid linkage was established by partial acid catalyzed degradation with phloroglucinol. Mild degradation afforded 64.0% (–)-EC-ph which coeluted with B2-ph ((–)-EC-4*β*→8-(–)-EC-ph), 10.8% of dimer B2 ((–)-EC-4*β*→8-(–)-EC), and 24.2% (–)-EC. A small amount of **1** (approx. 1.0%) remained intact. Taking this and the reaction products B2-ph and B2 into consideration, both interflavanoid bonds must be 4→8 linked. Consequently, **1** was confirmed to be procyanidin C1 (–)-EC-4*β*→8-(–)-EC-4*β*→8-(–)-EC.

The complete degradation of **2** yielded (–)-EC-ph and (+)-C. Mild phloroglucinolysis yielded 63.2% (–)-EC-ph and B2-ph, 11.3% dimer B1 ((–)-EC-4*β*→8-(+)-C), 25.1% (+)-C, and 0.4% unreacted trimer (**2**). Consequently, **2** was (–)-epicatechin-4*β*→8-(–)-epicatechin-4*β*→8-(+)-catechin [(–)-EC-4*β*→8-(–)-EC-4*β*→8-(+)-C].

Mild phloroglucinolysis indicated 40.7% (–)-EC-ph, 9.9% B5-ph ((–)-EC-4*β*→6-(–)-EC-ph), 12.4% dimer B2, 18.5% (–)-EC, and 18.5% unreacted trimer (**3**). The large amount of unreacted trimer (**3**) is an evidence for a 4→6 linkage. B5-ph indicates that the first interflavanoid bond is 4→6 linkage and the presence of dimer B2 reveals 4→8 linkage for the second.

Complete degradation of **4** gave (–)-EC-ph and (+)-C as the sole products, establishing that the procyanidin extension units possess (–)-EC and (+)-C as terminal unit. Mild degradation of **4** with phloroglucinol gave products that differed from those of **3** in only one entity, the presence of dimer B1 instead of dimer B2. This dimer B1 indicated the terminal unit was composed of (–)-EC-4*β*→8-(+)-C. Mild degradation of the trimer (**4**) gave 47.4% (–)-EC-ph, 9.8% B5-ph, 13.0% dimer B1, and 29.8% (+)-C, confirming **4** was (–)-epicatechin-4*β*→6-(–)-epicatechin-4*β*→8-(+)-catechin [(–)-EC-4*β*→6-(–)-EC-4*β*→8-(+)-C].

A full degradation showed two equivalents (–)-EC-ph for **5** and **6**, and one equivalent (–)-EC for **5** and (+)-C for **6** as terminal unit. The results of mild phloroglucinolysis yielded for **5**: 47.3% (–)-EC-ph and B2-ph, 28.4% dimer B5 ((–)-EC-4*β*→6-(–)-EC), 14.3% (–)-EC, and 10.0% unreacted trimer. Mild phloroglucinolysis of **6** yielded 45.0% (–)-EC-ph and B2-ph, 31.0% dimer B7 ((–)-EC-4*β*→6-(+)-C), 11.8% (+)-C, and 12.2% unreacted trimer. Hence, **5** was confirmed to be (–)-epicatechin-4*β*→8-(–)-epicatechin-4*β*→6-(–)-epicatechin [(–)-EC-4*β*→8-(–)-EC-4*β*→6-(–)-EC] and **6** was (–)-epicatechin-4*β*→8-(–)-epicatechin-4*β*→6-(+)-catechin [(–)-EC-4*β*→8-(–)-EC-4*β*→6-(+)-C].

Complete degradation of **7** afforded one equivalent (+)-C-ph, one equivalent (–)-EC-ph and one equivalent (–)-EC. Mild phloroglucinolysis gave 26.2% (+)-C-ph, 29.1% (–)-EC-ph, 13.2% B1-ph, 0.9% dimer B4 ((+)-C-4*α*→8-(–)-EC), and 30.6% (–)-EC. B1-ph and dimer B4, as well as traces of unreacted trimer, indicated that the two interflavanoid bonds are 4→8 linked. Moreover, these results indicated dimer B1 was present in the upper and dimer B4 in the lower unit as well as (–)-EC in the terminal unit. Hence, **7** was unambiguously identified as (–)-epicatechin-4*β*→8-(+)-catechin-4*α*→8-(–)-epicatechin [(–)-EC-4*β*→8-(+)-C-4*α*→8-(–)-EC].

On treatment with phloroglucinol under mild reaction conditions **8** yielded 25.7% (+)-C-ph, 27.8% (–)-EC-ph, 15.6% B1-ph, 1.9% dimer B3 ((+)-C-4*α*→8-(+)-C), 29.0% (+)-C, and unreacted trimer in traces. Hence, the data confirmed **8** as (–)-epicatechin-4*β*→8-(+)-catechin-4*α*→8-(+)-catechin [(–)-EC-4*β*→8-(+)-C-4*α*→8-(+)-C].

Compound **9** yielded 43.4% (+)-C-ph, 37.9% (+)-C, and 18.7% unreacted dimer (**9**) and compound **10** 41.8% (+)-C-ph, 35.8% (–)-EC, and 22.4% unreacted dimer (**10**). These results clearly showed the 4→6 interflavanoid bond in **9** and **10** and demonstrate that **9** is procyanidin dimer B6 ((+)-catechin-4*α*→6-(+)-catechin [(+)-C-4*α*→6-(+)-C]) and **10** B8 ((+)-catechin-4*α*→6-(–)-epicatechin [(+)-C-4*α*→6-(–)-EC]).

Figure Captions

Figure 3. Aromatic region of the 2D TOCSY spectrum of **1** at 240 K showing the assignment of the B-rings, where the upper, middle and terminal units are indicated as *u*, *m*, and *t,* respectively.

Figure 5. Schematic of selected through-bond and through-space correlations observed in the 2D COSY and ROESY spectra, respectively, that define the structure of **1**.

Figure 6. Exchange signals for **1** in the ROESY spectrum at 240 K in the region δ_H 4.5 – 5.5 show one major conformation.

Footnote: Rotation about each 4→8 interflavanoid bond lead to two minor conformers (b) for each major signal (a). The assignments of the upper, middle and terminal units are indicated as *u*, *m*, and *t,* respectively.

Figure 3

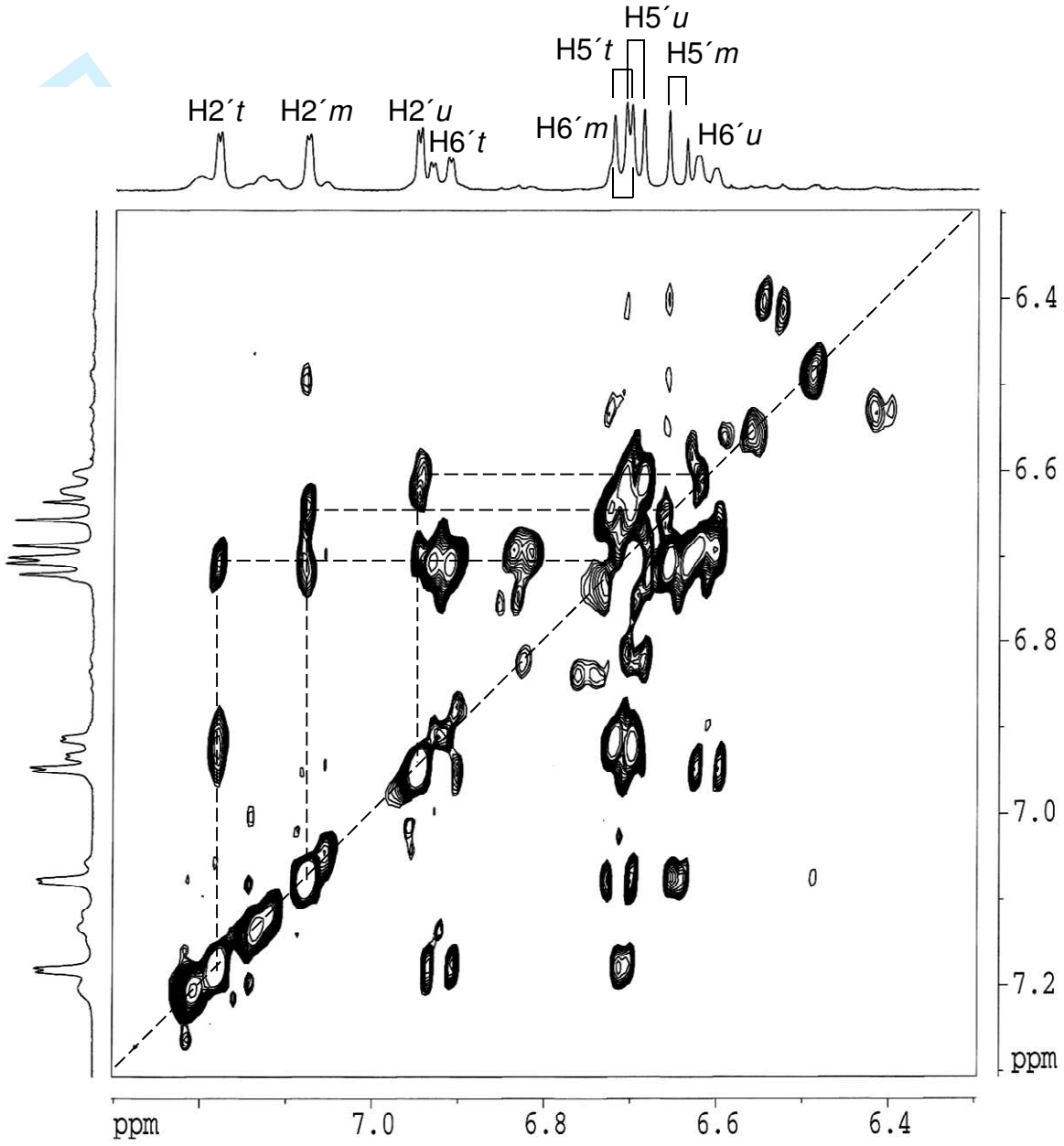

H5´u
H5´t
H5´m
H2´t
H2´m
H2´u
H6´t
H6´m
H6´u
6.4
6.6
6.8
7.0
7.2
ppm
ppm
7.0
6.8
6.6
6.4

Figure 5

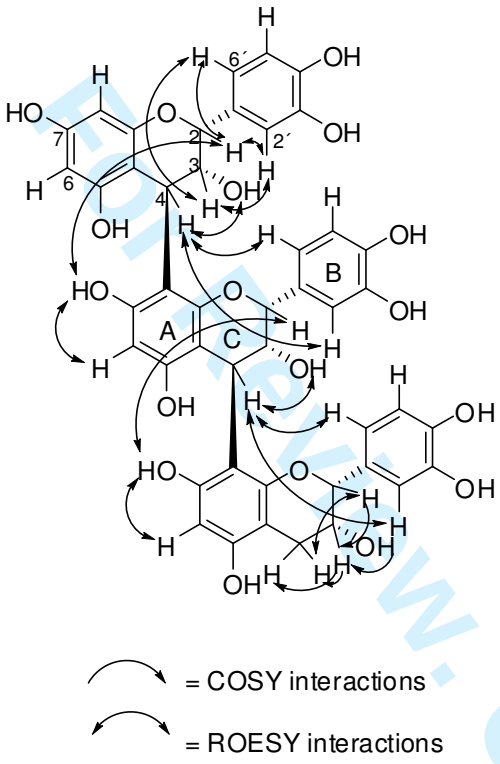
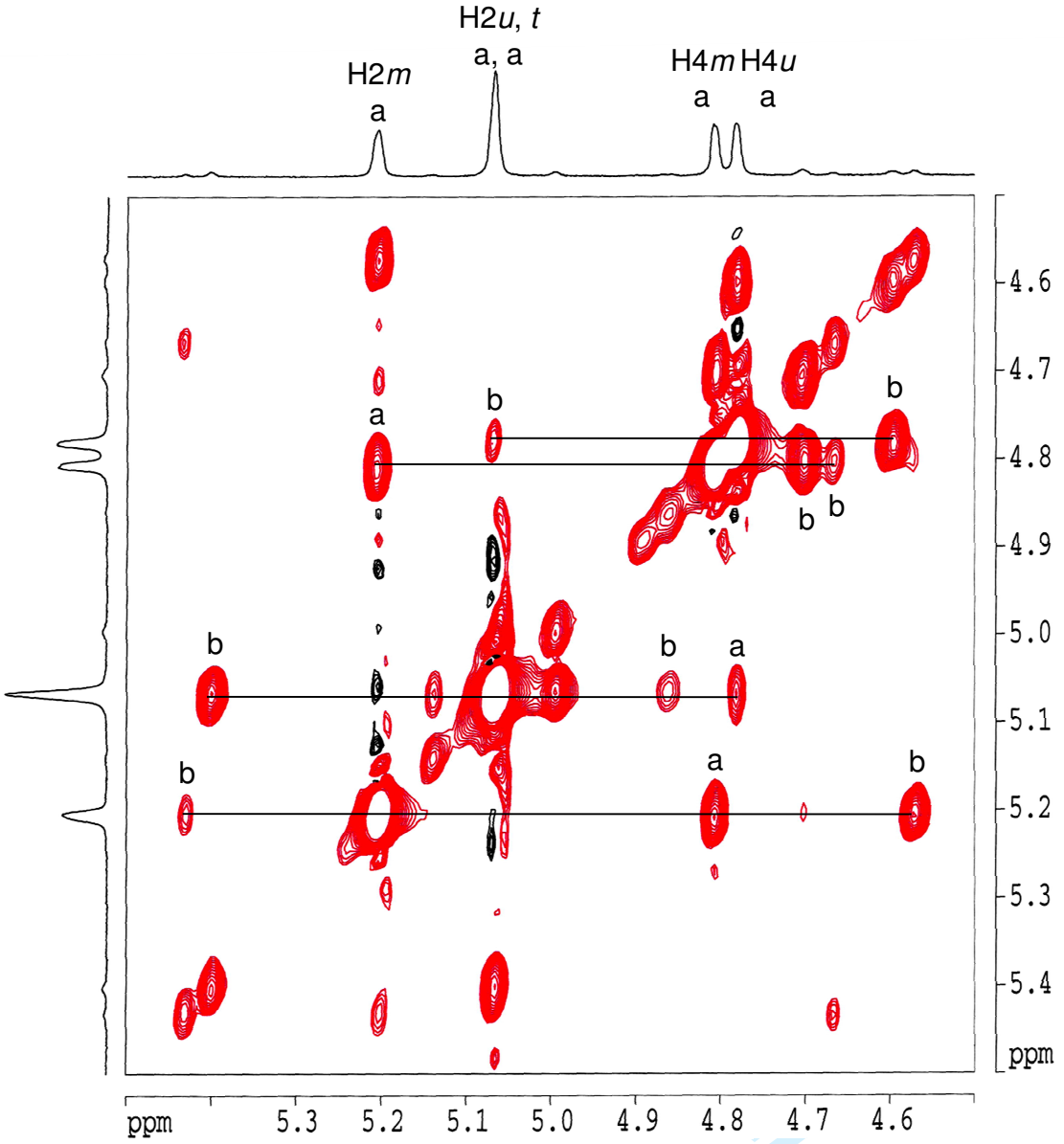


Figure 6


H2m
a
H2u, t
a, a
H4m H4u
a a
4.6
4.7
4.8
4.9
5.0
5.1
5.2
5.3
5.4
ppm
ppm
5.3
5.2
5.1
5.0
4.9
4.8
4.7
4.6