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New phenolic compounds obtained by evolution of (+)-catechin and glyoxylic acid in hydroalcoholic medium

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Abstract

The reaction between (+)-catechin **1** and glyoxylic acid in model solution system was investigated by LC/DAD and LC/ESI-MS analysis and the formation of new phenolic compounds **2**, **3**, **4** exhibiting absorption maxima near 300 nm were observed. The structures of these compounds were elucidated by ESI-MS and 1D and 2D NMR spectroscopy.

Keywords: phenolics; aldehydes; formylation; NMR.

Oxidative browning is one of the major causes of quality loss during storage of most fruit derived foods and beverages, and this reactivity raises an important economic question. Oxidation of polyphenols is considered as the major browning reaction which may be either catalyzed by specific enzymes like polyphenol oxidase (PPO) or due to autoxidation involving phenolic and non-phenolic compounds.[1–8] In particular, reactions of acetaldehyde and glyoxylic acid which result from oxidation of ethanol and tartaric acid, respectively, with phenolic compounds are believed to play an important role in wine browning.[9–11] The full characterization of these reactions has not been achieved directly in wine because of difficulties encountered in extracting and separating the brown pigments from food products, in which many reactions occur. Model solutions constitute then a simplified medium for their exploration allowing the detection of the newly formed compounds, their isolation and the elucidation of their structures.

In previous works, both enzymatic and non-enzymatic oxidation of (+)-catechin were shown to produce different colourless and yellowish adducts.[11–16] This work describes the detection and the isolation of new phenolic compounds **2**, **3**, **4** (Fig. 1), formed by interaction between (+)-catechin **1** and glyoxylic acid in hydroalcoholic medium. Their characterization by ESI-MS and 1D and 2D NMR techniques is also presented.

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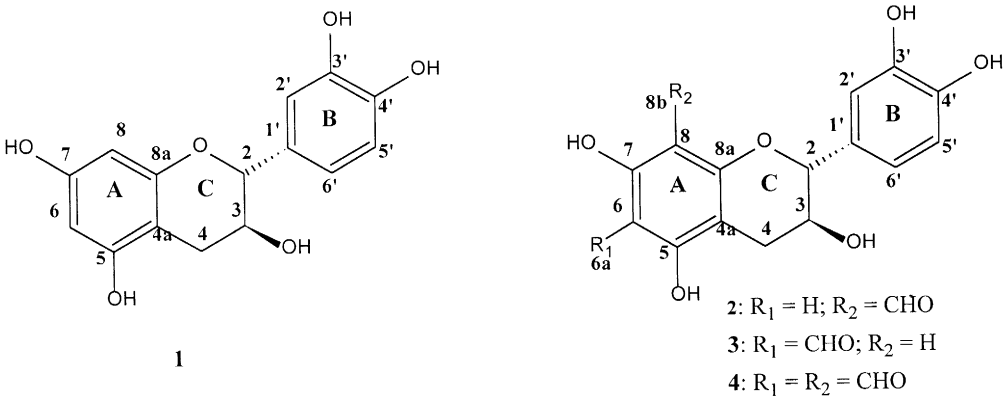

Fig. 1.

The reaction of (+)-catechin and glyoxylic acid was investigated as a model of browning phenomenon which is generally observed during ageing of grape derived foods. The originally colourless solution became brown with time, and a rapid decrease in the concentration of (+)-catechin concomitant with the appearance of new products in the mixture was observed by LC/DAD analysis. Among the obtained compounds, the four previously described dimeric derivatives in which two (+)-catechin units are linked to each other by carboxymethine group were detected.[11] The role of these colourless compounds as intermediates in the reaction pathways leading to brown pigments has been demonstrated earlier.[14–16] In addition to these compounds, three new compounds with maximum absorption at 295 nm and a shoulder around 340 nm were observed (Fig. 2).

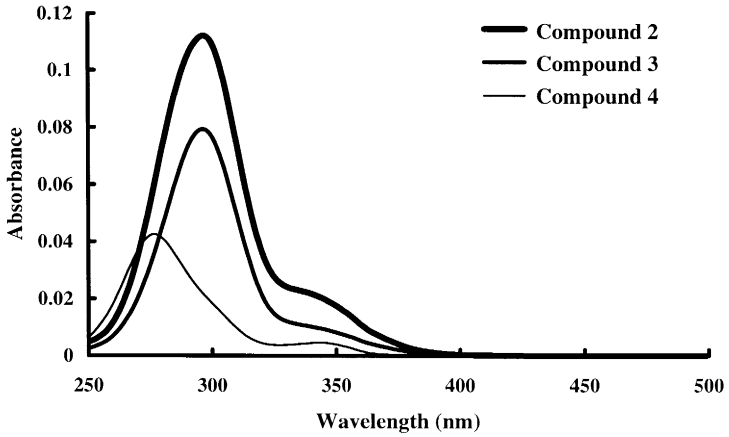

Fig. 2. UV–visible spectra of compounds **2**, **3** and **4**

These compounds were isolated by HPLC at the semipreparative scale and submitted to further investigations in order to elucidate their structures. LC/ESI-MS analysis of compounds **2** and **3**, conducted in the negative ion mode, showed a signal at *m/z*=317 for both compounds corresponding to a $[M-H]^-$ ion, indicating a molecular weight of 318 and an additional 28 uma with respect to (+)-catechin. Complete structure elucidation of compound **2** was further achieved by 1D and 2D NMR analyses at 500 MHz in DMSO:TFA (9:1).

The usual pyrane ring protons were assigned by ^{1}H and HSQC experiment. The three B ring protons were observed between 6.60 and 6.74 ppm. From the aromatic A ring, only one proton signal appearing as a singlet at 5.93 ppm was present, indicating a probable A ring substitution. An additional singlet at 9.91

ppm, showing in HSQC experiment, a correlation with a carbon at 191.1 ppm, suggesting the presence of an aldehyde function. The structure of compound **2** was then concluded to consist of a (+)-catechin moiety linked via its C-6 or C-8 to an aldehyde group.

Its definitive structure was elucidated by HMBC[17] experiment which allowed assignment of all hydrogen and carbon atoms (Table 1). The doublet at 4.84 ppm was easily attributed to the H-2 of the C ring due to its correlation in HSQC with the carbon at 81.7 ppm. In addition to its long range correlations with the C-3, C-4 and C-1′, this proton showed a correlation with a signal at 158.0 ppm which corresponds to the chemical shift of an oxygenated aromatic carbon which can only be C-8a. The aldehydic proton showed correlations with C-7, C-8 and C-8a suggesting a C-8 substitution. This was also confirmed by the presence of correlations between the residual aromatic proton and the two oxygenated aromatic carbons located at 165.4 and 163.4 ppm, attributed, respectively, to C-5 and C-7. The other quaternary carbons were assigned by their long range correlations observed in the HMBC spectrum as shown in Fig. 3.

Table 1
^{1}H and ^{13}C assignments of compounds **2**, **3** and **4** in DMSO-d_6–TFA (9:1)

Position	**2**		**3**		**4**	
	δ ^{1}H (ppm); *m*; *J* (Hz)	δ^{13}C	δ ^{1}H (ppm); *m*; *J* (Hz)	δ^{13}C	δ ^{1}H (ppm); *m*; *J* (Hz)	δ^{13}C
2C	4.84; *d; J* = 6.3	81.7	4.78; *d; J* = 6.3	82.1	5.08; *d; J* = 5.5	82.6
3C	3.98; *dd; J* = 6.7, 16.0	65.6	3.96; *dd; J* = 6.7, 16.0	65.6	4.30; *m*	64.3
4Cα	2.41; *dd; J* = 5.2, 16.6	26.2	2.42; *dd; J* = 5.2, 16.6	25.5	2.50; *m*	23.9
4Cβ	2.53; *m*	26.2	2.56; *m*	25.5	2.50; *m*	23.9
5A	-	165.4	-	163.2	-	168.5
6A	5.93; *s*	94.7	-	105.4	-	103.7
7A	-	163.4	-	161.2	-	167.5
8A	-	104.3	6.20; *s*	94.2	-	103.4
4aA	-	100.3	-	99.4	-	100.0
8aA	-	158.0	-	163.0	-	163.4
1’B	-	130.0	-	130.0	-	128.8
2’B	6.74; *d; J* = 1.8	114.2	6.67; *bs*	114.3	6.71; *m*	113.5
3’B	-	145.4	-	145.4	-	145.8
4’B	-	145.4	-	145.4	-	145.8
5’B	6.70; *bd; J* = 8.1	115.7	6.70; *bd; J* = 8.1	115.7	6.71; *m*	115.4
6’B	6.60; *dd; J* = 8.1, 1.8	118.1	6.56; *bd; J* = 8.1	117.6	6.60; *dd; J* = 1.8, 8.2	117.6
6a	-	-	9.97; *s*	191.9	10.06; *s*	191.5
8b	9.91; *s*	191.1	-	-	9.99; *s*	191.9

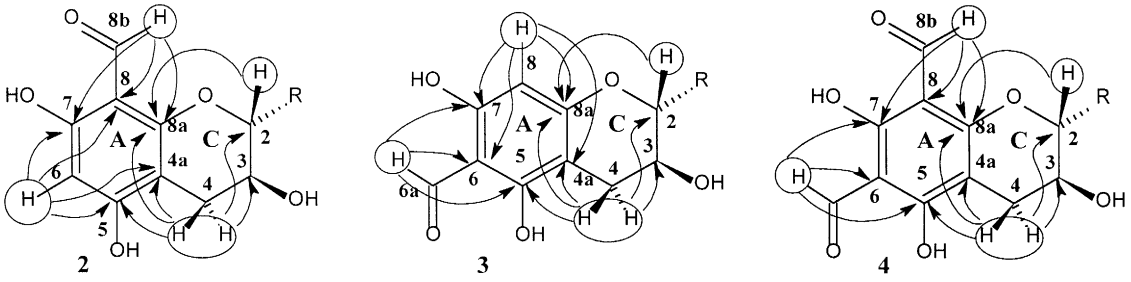

Fig. 3. Main HMBC correlations observed in compounds **2**, **3** and **4**

The same analysis was applied to compound **3**. In its HMBC spectrum the H-2 proton doublet at 4.78 ppm correlated with the signal at 163.0 ppm which corresponds thus to C-8a. This carbon showed correlation with the residual aromatic proton located at 6.20 ppm which was then attributed to H-8, indicating a 6 substitution. This was confirmed by the presence of correlations between C-5, C-6 and C-7 carbons and the aldehydic proton (Fig. 3). Compound **3** was then concluded to be the isomer in which the aldehydic group is linked to the C-6 carbon.

The UV–visible spectrum of compound **4** showed a maximum around 280 nm and an accentuated shoulder at 350 nm (Fig. 2). Its LC/ESI-MS analysis in the negative ion mode showed a molecular weight of 346 indicating another additional 28 amu compared to those observed for compounds **2** and **3**. This could be due to the presence of two aldehydic moieties and was further confirmed by 1D and 2D NMR analysis. Thus in the ^{1}H NMR spectrum of compound **4**, no A ring aromatic proton signal was observed in agreement with the disubstitution hypothesis. This was also supported by the presence of two singlets located, respectively, at 9.99 and 10.06 ppm and assigned to the two aldehydic protons. In HSQC experiment, these two protons showed correlations with two carbons located, respectively, at 191.9 and 191.5 ppm corresponding to the two carbonyl functions. The definitive structure of compound **4** was determined on the basis of HMBC experiment which allowed complete assignment of proton and carbon chemical shifts (Table 1 and Fig. 2).

The carbon C-8a was assigned to the signal at 163.4 ppm through its correlation with the H-2 proton assigned to the doublet at 5.08 ppm. This carbon showed in HMBC experiment a correlation with the aldehydic proton located at 9.99 ppm, indicating thus an 8 substitution. On the other hand, the signal at 168.5 ppm was assigned to the C-5 carbon through its correlation with the H-4 protons. This carbon showed a correlation with the aldehyde proton located at 10.06 ppm indicating, thus, a 6 substitution and confirming the 8 substitution.

This study showed thus the formation of a new type of phenolic compounds by evolution of (+)-catechin and glyoxylic acid in hydroalcoholic solution medium. Their structure was shown to consist of a (+)-catechin substituted by aldehydic units through the C-6 and/or C-8 carbon atoms. The formation of compound **2** (as an example) may involve the first formation of the intermediate **5**, which by loss of a formic acid molecule gave the aldehydic derivative (Fig. 4). The formation of such compounds in model solution system indicates their probable contribution in colour transformation of fruit derived foods. In addition, the presence of an aldehydic moiety in their structure allows them to play a role of polymerizing agent like it was observed in the case of acetaldehyde or glyoxylic acid.[9,11] Our results also indicate that other reaction pathways contributing to the formation of highly polymerized compounds which finally precipitate and to browning compete with the previous polycondensation reactions.

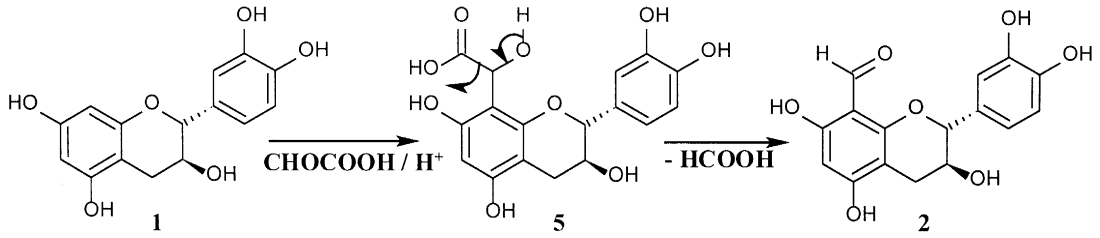

Fig. 4. Mechanism of compound **2** formation from (+)-catechin **1** via the intermediate **5**

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