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Synthesis of a new catechin-pyrylium derived pigment

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Abstract—A new catechin-pyrylium derived pigment compound was synthesised from the reaction of catechin with sinapaldehyde in acidic conditions and its structure has been characterised by UV–vis, MS and NMR spectroscopy. Similar compounds could be expected to be formed in wines and contribute to changes in colour and astringency during ageing.

Condensed tannins (i.e., proanthocyanidins) constitute a group of polyphenols widespread in the plant kingdom. In foodstuffs such as red wine, these compounds contribute to taste (e.g., astringency) and also to the colour change during ageing.[1] Effectively, catechins may form noncovalent interaction with anthocyanins (red pigments) through co-pigmentation phenomena increasing the colour intensity and promoting a bathochromic shift of the λ_{max} in the visible region of the anthocyanin spectra.[2,3] Besides, catechins may react with anthocyanins, either directly or involving aldehydes giving rise to several types of anthocyanin derived pigments such as catechin–anthocyanin alkyl linked adducts and pyranoanthocyanin–catechin derivatives with different colours.[4–9] Finally, catechin may also react with aldehydes yielding yellowish xanthylium salts.[1,10]

Wine storage in oak barrels during the first years of ageing is a procedure widely employed by winemakers in order to improve quality. During this period the wood releases aldehydes (e.g., furfural, hydroxymethylfurfural, hydroxybenzaldehyde, vanillin, sinapaldehyde) and other compounds (e.g., ellagitannins) to the wine, which directly contribute to wine aroma and taste. Aldehydes released from oak barrels are also expected to react with polyphenols and play a crucial important role in the achievement of a better and stable wine colour.

In this letter, the synthesis of a new brick-red catechin-pyrylium derived pigment **1** (Fig. 1) from the reaction of catechin **2** with sinapaldehyde **3** is described and its chemical structure elucidated.

Catechin **2** (1 mM) was incubated in 20% aqueous ethanol, pH 3.2, at 35 °C with sinapaldehyde **3** (10 mM). The appearance of the catechin-pyrylium salt (**1**) was monitored by HPLC-DAD on a 250 × 4.6 mm i.d. reversed-phase C18 column. The elution system consists of two solvents: A, water/formic acid (95:5); B, acetonitrile.

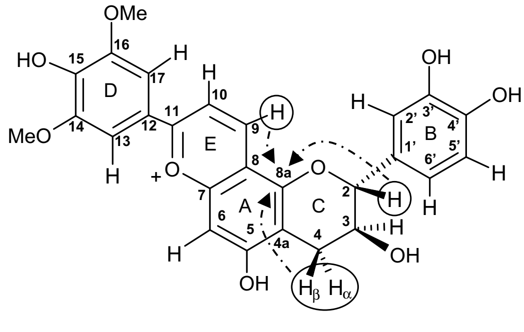

Figure 1. Structure of the new synthesised catechin-pyrylium salt (**1**).

Keywords: Catechin; Aldehyde; Sinapaldehyde; Tannin; Catechin-pyrylium; Red colour.

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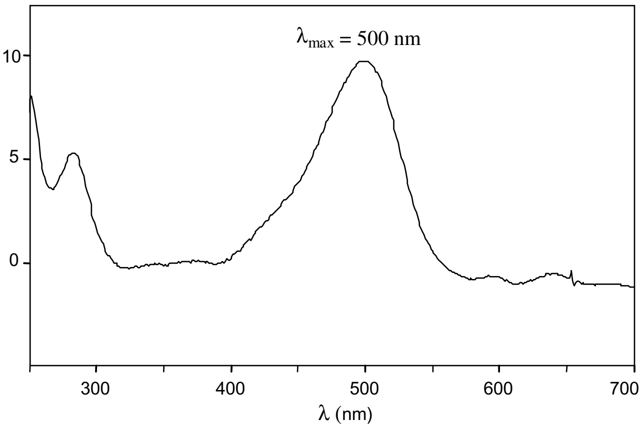

Figure 2. UV–vis spectrum of the new catechin-pyrylium salt (**1**).

The elution gradient was performed from 10% to 35% B for 50 min at a flow rate of 1.5 mL/min.

The formation of a new compound was observed that revealed a maximum absorption in the UV–vis spectrum at 500 nm, conferring it a brick-red colour (Fig. 2).

The mass spectrum of this compound obtained by LC–DAD/ESI/MS in the positive ion mode showed a molecular ion $[M]^+$ at *m/z* 479. In addition two major fragment ions were detected at *m/z* 327 and 357 in the respective MS^2 spectrum. The fragment $[M-152]^+$ at *m/z* 327, corresponds to a retro-Diels–Alder of the catechin moiety.

The structure of this new catechin-pyrylium salt was further elucidated by ^1H and ^{13}C NMR spectroscopy using 2D techniques (COSY, HSQC, HMBC)[11–13] (Table 1).

Concerning the catechin moiety, the two protons H-4αC and H-4βC were located at δ 2.80 and 2.95 ppm, respectively, through the characteristic ABMX spin system of the pyran ring C observed in the COSY spectrum. Proton H-3C was assigned to the multiplet at 4.23–4.31 ppm from its correlations with protons H-4αC (weak) and H-4βC (strong). H-3C also correlates with H-2C, which was thus assigned to the doublet at 5.13 ppm (J = 6.5 Hz). This latter outcome suggests a *trans* configuration relative to H-3C, which is characteristic of (+)-catechin.[14] The only proton detected on ring A (H-6A) was assigned to the singlet at δ 7.11 ppm. For the protons of ring B, H-2′B was assigned to a broad singlet at δ 6.84 ppm, and protons H-5′B and H-6′B were attributed to the doublets (J = 8.1 Hz) at δ 6.80 and at δ 6.75 ppm, respectively.

The protons H-13D,17D and the two methoxyl groups of ring D were assigned to the singlets at δ 7.67 and 4.01 ppm, respectively.

The vinylic protons H-9E and H-10E that revealed a clear correlation in the COSY spectrum were attributed to the two doublets located at δ 9.04 and 8.18 ppm (J = 8.8 Hz), respectively.

The assignment of the carbon resonances was possible using two-dimensional NMR techniques (HSQC and HMBC). The correlation observed between the resonances of H-13D/H-17D and that of the carbons at δ 148.9 and 145.3 ppm allowed their assignment to C-14D,16D and C-15D, respectively. Carbons C-12D and C11E were assigned at δ 118.9 and 170.7 ppm, respectively, through their HMBC correlations with both H-13D,17D and H-10E protons. Carbons C-9E and C-10E were assigned at 146.6 and 110.1 ppm through HSQC correlation with the respective protons.

Carbons C-4aA and C-7A were determined from their long range ^1H–^{13}C correlation with H-6A. The quaternary carbons C-5A and C-8aA were assigned at δ 168.3 and 152.8 ppm from their long distance correlations with protons H-4C observed in the HMBC spectrum. Carbon C-8aA was distinguished from carbon C-5A from its HMBC correlation with protons H-2C and H-9E (Fig. 1). The vinylic proton H-9E showed long distance ^1H–^{13}C correlations with carbon 8aA.

Table 1. ^1H (500.13 MHz) and ^{13}C (125.77 MHz) NMR spectral data of the new catechin-pyrylium salt **1**, in CD_3OD/TFA (98:2)

Position	δ^1H (ppm); J (Hz)	δ ^{13}C	Position	δ^1H (ppm); J (Hz)	$\delta^{13}C$
Catechin moiety			D ring		
2C	5.13; d, 6.5	82.7	12D		118.9
3C	4.22–4.31; m	65.2	13D/17D	7.67; s	106.5
4αC	2.95; dd, 4.8, 17.3	25.9	14D/16D		148.9
4βC	2.80; dd, 6.7, 17.3	25.9	15D		145.3
4aA		110.1	14-OMe/16-OMe	4.01; s	55.5
5A		168.3			
6A	7.11; s	94.3	E ring		
7A		157.1	9E	9.04; d, 8.8	146.6
8A		111.6	10E	8.18; d, 8.8	110.1
8aA		152.8	11E		170.7
1′B		128.8			
2′B	6.84; br s	113.0			
3′B		145.3			
4′B		145.3			
5′B	6.80; d, 8.1	114.8			
6′B	6.75; d, 8.1	117.9			

These correlations allowed the unambiguous identification of the position of the pyrylium ring E linkages onto the carbons C-7A and C-8A. These correlations would unlikely be observed if the ring E was formed between the hydroxyl group at carbon C-5A and the carbon C-6A.

Carbon resonances of C-2′, C-5′ and C-6′ of ring B were assigned at δ 113.0, 114.8 and 117.9 ppm, from their direct ^{1}H–^{13}C correlations (HSQC) with H-2′B, H-5′B and H-6′B, respectively. Carbons C-3′B and C-4′B were assigned at δ 145.3 ppm from their long distance ^{1}H–^{13}C correlations (HMBC) with protons of ring B.

Finally, carbon C-1′B was assigned at δ 128.8 ppm from its long range ^{1}H–^{13}C correlation with H-2′B, H5′B and H-2C. Carbon resonances of C-2, C-3 and C-4 of ring C were easily assigned at δ 82.7, 65.2 and 25.9 ppm from direct ^{1}H–^{13}C correlations in the HSQC spectrum.

The formation mechanism of compound **1**, in acidic medium, is thought to firstly involve the protonation of sinapaldehyde **3**, with the formation of the respective carbocation followed by the nucleophilic addition of C-8A of the catechin **2** (Fig. 3). The dehydration of the resulting protonated adduct yields a new carbocation,

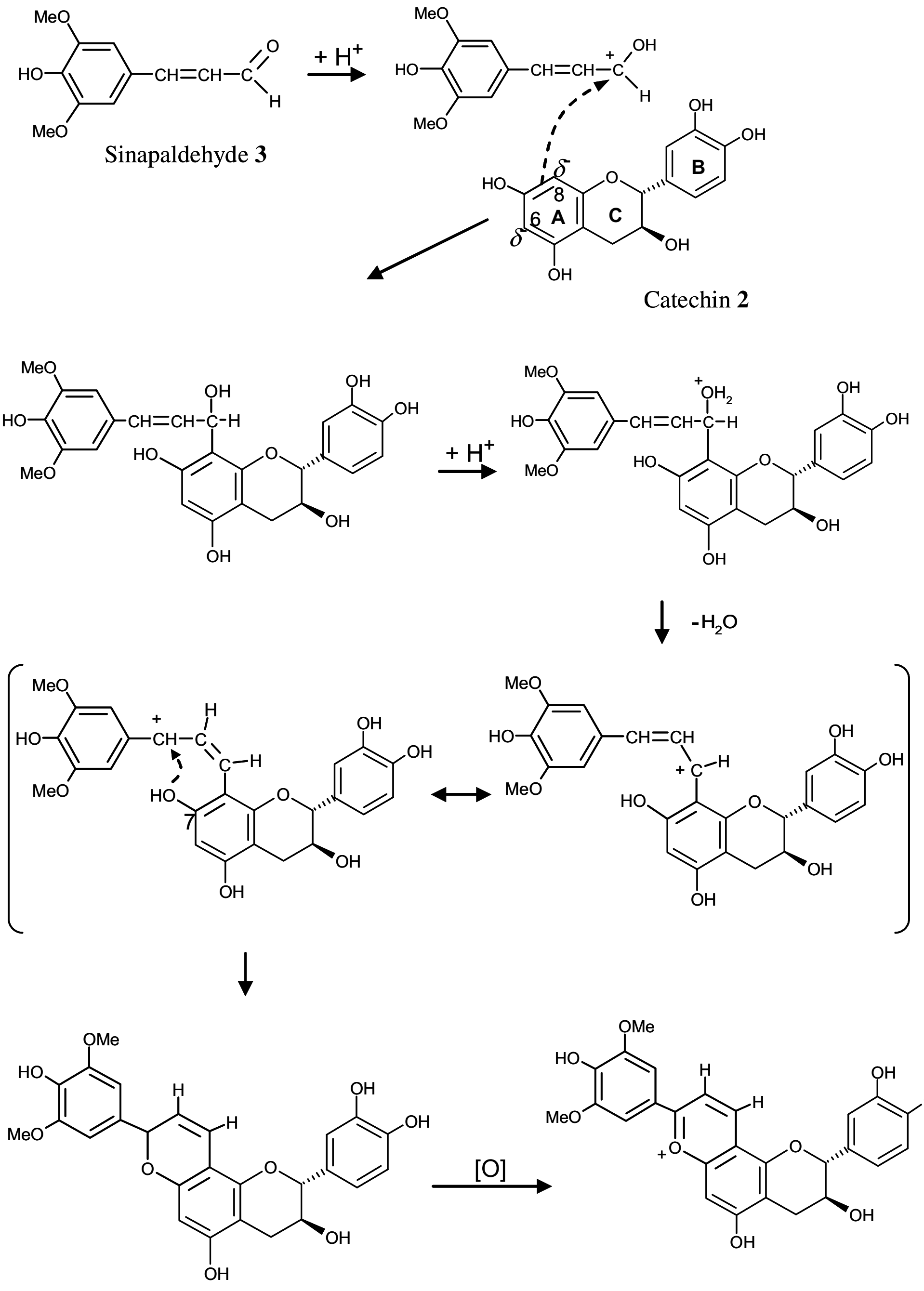

Figure 3. Hypothetic mechanism for the formation of catechin-pyrylium pigment **1** obtained from catechin **2** and sinapaldehyde **3**.

which suffers an intra-molecular nucleophilic attack by the hydroxyl group at carbon 7 of ring A. The final oxidation yield the pyrylium ring E that associated with the aromatic ring A constitutes a chromophore group. The extended conjugation of the π electrons are at the origin of their maximum absorptivity at 500 nm.

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