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Structure of a New Xanthylium Salt Derivative

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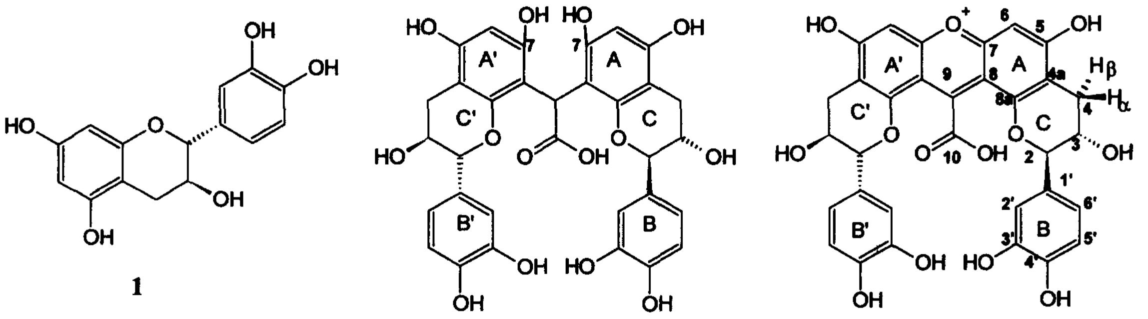
Abstract. A new polyphenolic compound with a xanthylium skeleton has been synthesised from (+)-catechin and glyoxylic acid. Its structural elucidation was achieved by UV, MS and NMR spectroscopies. Its formation involved glyoxylic acid-mediated dimerisation of (+)-catechin **1** giving thus compound **2**, followed by cyclisation to a xanthene derivative **3** which by oxidation afforded the xanthylium compound **4**. The detection and structural determination of the xanthene compound confirmed the postulated mechanism. © 1999 Published by Elsevier Science Ltd. All rights reserved.

Flavanols are polyphenolic compounds found in many plants, fruits and beverages such as fruit juices, beer and wine. They have attracted much attention in relation to their physiological activities and their role has become an important issue in the relationship between health and human diet. During conservation, phenolic compounds usually undergo progressive changes which affect sensorial properties like colour, taste and colloidal stability[1,2]. Various mechanisms have been suggested to explain such transformations. Processes involving either direct condensation between flavanols and anthocyanins giving rise to compounds with a yellow brown hue[3,4] (xanthylium salts) or reactions mediated by acetaldehyde with the formation of violet pigments [5-11] have been studied in model solution systems. While the process involving acetaldehyde is well documented, little is known about the formation and the structures of xanthylium salts. However, it is believed that xanthylium nuclei might occur in condensed tannins (flavanol polymers), and that such chromophores may contribute to the high absorbance of wine pigments in the 400-500 nm region[4,12,13]. In this paper, we describe the synthesis, the isolation and the chemical structure analysis of a new yellowish xanthylium compound named NJ2 **4** formed by interaction between (+)-catechin **1** and glyoxylic acid (figure 1).



1 2 4

Figure 1

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Incubation of (+)-catechin 1 with tartaric acid in iron catalytic medium yielded colourless compounds exhibiting maxima at 280 nm and yellowish compounds with maxima in the region of 440 - 460 nm as reported earlier[14,15]. As previously indicated[14], the obtained colourless compounds consisted of (+)-catechin units bridged by a methine carboxylic acid group (compound 2). Formation of the yellowish compounds was related to the disappearance of the colourless ones suggesting that the former were probably formed by evolution and further rearrangement of the latter. This prompted us to prepare them from individually isolated colourless products.

Thus, the major colourless dimer 2 was isolated by high performance liquid chromatography at the semipreparative scale and was further incubated in pH 3.5 solution, and appearance of two new compounds referred as to NJ2 and NJ3, initially absent in the mixture were observed. Their retention times and UV-visible spectra showing absorption maxima around 440 and 460 nm, respectively are the same as those of pigments formed in reaction between (+)-catechin and tartaric acid in iron catalysed medium[15].

LC/ESI-MS analysis of the major yellowish compound NJ2 both in positive and negative ion modes showed *m/z* values at 615 and 617, respectively. The fact that the molecular weight of compound NJ2 was 20 mass units lower than that of the colourless dimer 2 ($[M-H]^-$ at *m/z* = 635), suggested that the yellowish compound may be formed by a dehydration followed by an oxidation process. The dehydrated product (Mr = 618) was actually detected by LC/MS analysis at *m/z* 617, in the negative ion mode. The loss of a water molecule may be achieved either between the carboxyl group and a neighbouring hydroxyl group like those at the 7 position of the A or A' rings yielding, after oxidation, a lactonised product, or between the two 7-OH groups, giving a xanthylium salt 4 after an oxidation process.

The UV-visible spectrum of NJ2 showed two maxima at 273 and 444 nm in addition to a shoulder at 308 nm. These maxima were bathochromically shifted to 283, 496 and 325 nm by addition of NaOH (figure 2), as reported earlier for xanthylium salts[4,13,16].

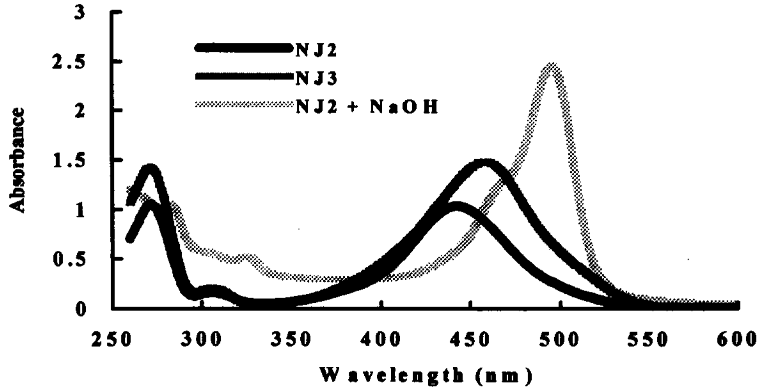

Figure 2: UV-Visible spectra of compounds NJ2, NJ3 and NJ2 + NaOH.

The fragment ions at 571, 463 and 419 obtained by mass spectroscopy, in the negative ion mode, can be attributed respectively to the loss of carboxyl residue (-44 mass units), the loss of a hydroxyvinylphenol group ($C_8H_8O_3$)obtained by retro-Diels-Alder fission (-152 mass units) and finally the ion obtained after both fragmentations (-196 mass units), in agreement with the proposed structure 4.

The structure of NJ2 was further elucidated from ^{1}H and ^{13}C chemical shift assignements (Table 1). Unlike that of its colourless precursor which was reported to give two catechin systems[14], the ^{1}H spectrum of compound NJ2 presented only 8 protons, corresponding to one catechin spin system, thus indicating the existence of some symmetry in the structure. This was confirmed by 1D ^{13}C NMR spectrum, which showed only

17 signals (15 corresponding to one catechin skeleton and 2 constituting the bridge moiety). Besides, the disappearance of the proton signal previously attributed to that of the methine bridge was observed[14], in agreement with the proposed oxidised structure.

Position	δ ^{1}H (ppm); *m*; *J* (Hz)	δ ^{13}C
2C, 2C'	5.16; broad *d*; *J* = 3.3	82.7
3C, 3C'	4.18; *m*	64.4
4αC, 4αC'	2.46; *dd*; *J* = 3.6, 17.35	24.5
4βC, 4βC'	2.66; *dd*; *J* = 3.3, 17.35	24.5
4aA, 4aA'	-	107.9
5A, 5A'	-	170.5
6A, 6A'	6.90; *s*	95.6
7A, 7A'	-	156.8
8A, 8A'		104.8
8aA, 8aA'	-	154.9
9D	-	149.4
10	-	166.1
1'B, 1'B'	-	128.9
2'B, 2'B'	6.66; broad *s*	113.5
3'B, 3'B'	-	145.2
4'B, 4'B'	-	145.3
5'B, 5'B'	6.65; broad *d*; *J* = 8.0	115.7
6'B, 6'B'	6.54; broad *d*; *J* = 8.0	117.0

Table 1. ^{1}H and ^{13}C assignements of compound 4 in DMSO-d_6—TFA (9:1)

The signals which resonate at 2.46, 2.66, 4.18 and 5.16 ppm could be readily assigned to H-4, H-3 and H-2 of the C and C' rings, while those located at 6.65 and 6.54 ppm were assigned to H-5' and H-6' after COSY experiment. Assignement of the two remaining signals (6.66 and 6.90 ppm) was achieved using TOCSY experiment. The broad singlet located at 6.66 ppm wich correlates with both H-5' and H-6' was thus attributed to H-2' while the singlet at 6.90 ppm which gives no scalar correlation was assigned to H-6.

The ROESY spectrum also showed that protons H-2 and H-3 gave correlations with only two aromatic protons : one at 6.66 ppm which was already attributed to proton H-2', and the other at 6.54, which can be either H-5' or H-6'. Since the latter is in a more favourable position to give such coupling, the signal at 6.54 ppm was attributed to H-6', whereas the broad aromatic doublet resonating at 6.65 ppm was assigned to H-5'.

After the proton resonances had been assigned, all the corresponding carbons were attributed from the short-range HSQC experiment. The assignment of quaternary carbons was obtained from a long-range HMBC experiment. On the basis of these analyses, attributions of the various proton and carbon chemical shifts were achievcd, allowing to establish that the synthesised compound NJ2 was xanthylium salt 4.

From a mechanistic point of view, the formation of compound 4 may result from cyclisation of the colourless dimer giving a xanthene structure as previously reported for 9-methyl-xanthene[17]. The obtained xanthene 3 is then oxidised to xanthylium salt as shown in figure 3. The detection of the xanthene 3 compound (Mr: 618) among the products formed from NJ2 precursor 2 by LC-MS analysis confirmed thus the proposed mechanism. Moreover the intermediate xanthene derivative was obtained by reduction of the xanthylium NJ2 and its structure elucidated by UV, MS and NMR spectroscopies.

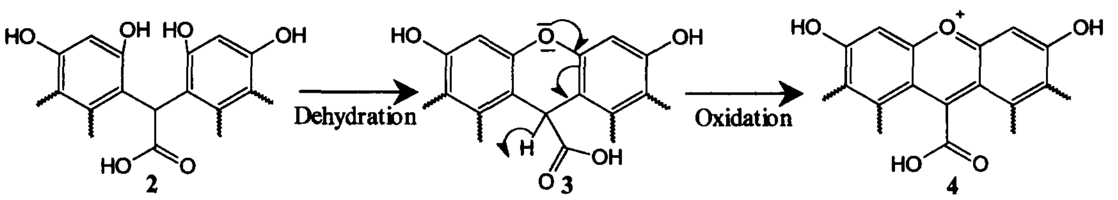

Figure 3: Mechanism of xanthylium 4 formation from the colourless dimer 2 via the xanthene 3 derivative.

Compared to the results obtained with the xanthylium salt 4, an additional singlet was observed at 4.86 ppm in the ^1H NMR spectrum and attributed to the proton H-9. This proton correlated, in HSQC experiment, with a carbon located at 34.45 ppm which was then attributed to C-9. In the HMBC experiment spectrum, correlations with C-7, C-8 and C-10 were observed, confirming the structure of the xanthene compound and thus offering another argument to support the proposed structure 4 for the yellow compound NJ2.

The formation of such yellow xanthylium compounds in wine-like model solutions suggests their possible contributions in colour evolution and browning observed during conservation and ageing of grape derived foods. In addition, more polymerised compounds where xanthene and xanthylium nuclei are incorporated were also detected in model solution system containing (+)-catechin and glyoxylic acid, after 24 hour incubation. This indicates the implication of such derivatives in the polymeric pigments responsible for the high absorption around 450 nm formed during wine ageing.

Our results also indicate that other reaction pathways contributing to browning compete with polycondensation reactions and offer new information and support to the contribution of xanthylium salts in colour evolution and browning. They finally open perspectives for further investigations of similar compounds. A number of properties such as temperature stability and copigmentation, in addition to their possible use as food colorants, remain of a high interest.

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