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Tetrahedron Letters 45 (2004) 3455–3457
Tetrahedron Letters

NMR structure characterization of a new vinylpyranoanthocyanin–catechin pigment (a portisin)

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Received 3 November 2003; revised 25 February 2004; accepted 3 March 2004

Abstract—A new polyphenolic compound, the structure of which corresponds to a pyranomalvidin-3-glucoside linked to a (+)-catechin unit through a vinyl linkage, has been characterized by UV–vis, MS and NMR spectroscopy. This compound was obtained in an aqueous alcoholic solution from the reaction between a malvidin-3-glucoside–pyruvic acid derivative and (+)-catechin in the presence of acetaldehyde.

Anthocyanins constitute a group of polyphenols widespread in the plant kingdom and extensively present in our diet. These compounds are responsible for the colour of many foodstuffs and beverages, such as red wine.[1,2] Anthocyanins contribute highly to the red-purple colour of young red wines, and during ageing undergo several transformations yielding new anthocyanin-derived structures.[3–5] These newly formed pigments present different chromatic features and exhibit different colours from orange-like to more bluish hues.[6] Recently, a new family of vinylpyranoanthocyanins, named portisins, was found to occur in aged port wine,[7] which exhibit a bluish colour in solution at acidic pH values.

In this paper, the synthesis of a portisin pigment **3** (Scheme 1) as a result of the reaction between a malvidin-3-glucoside–pyruvic acid derivative **1** and (+)-catechin **2** in the presence of acetaldehyde is described and its chemical structure elucidated.

The previously isolated and characterized[8] malvidin-3-glucoside–pyruvic acid derivative **1** was incubated in 20% aqueous ethanol, pH 1.5, at 35 °C with (+)-catechin **2** (molar ratio 1:100) in the presence of acetaldehyde (molar ratio of catechin/acetaldehyde 2:1) and the reaction was monitored by HPLC–DAD analysis. After reacting for 3 days, a new pigment initially absent in the mixture was detected by HPLC. Its UV–vis spectrum (Fig. 1) revealed an absorption maximum at 572 nm, quite bathochromically shifted from that of the malvidin-3-glucoside–pyruvic acid derivative **1** (511 nm).

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 831. In addition two major fragment ions were detected at m/z 669 and 517 in the respective MS^2 spectrum. The fragment $[M-162]^+$ at m/z 669, corresponds to the pigment aglycon as a result of the loss of the glucose moiety, whereas the fragment $[M-314]^+$ at m/z 517 corresponds to the loss of the glucose and a retro-Diels–Alder fission of the catechin moiety.

The structure of this portisin was further elucidated by ^1H and ^{13}C NMR using 1D and 2D techniques (COSY, NOESY, HSQC, HMBC)[9–11] (Table 1).

The protons H-2′B and H-6′B and the two methoxyl groups of ring B were situated at 7.60 and 3.92 ppm, respectively. The protons H-6A and H-8A of ring A

Keywords: Anthocyanin; Flavanol; Catechin; Portisin; Pyruvic acid; Blue colour.
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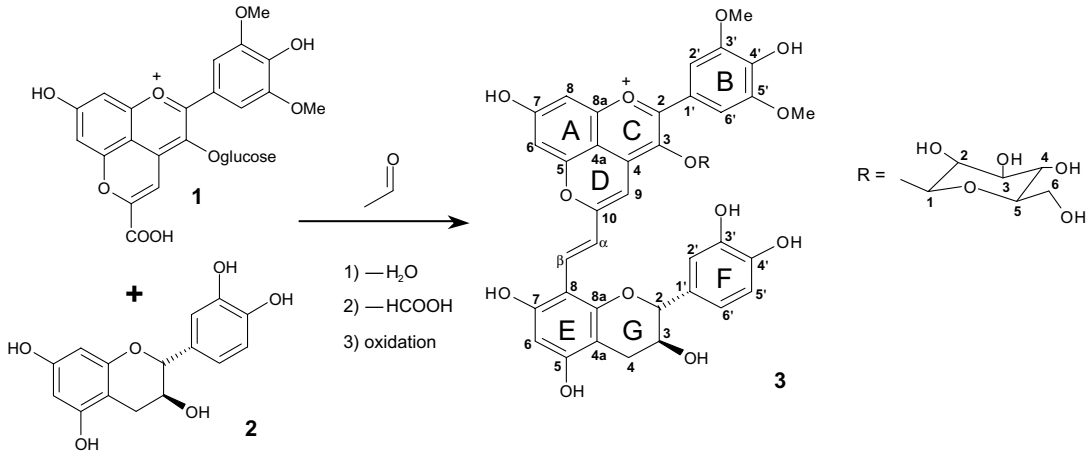
doi:10.1016/j.tetlet.2004.03.007



Scheme 1. Formation of vinylpyranomalvidin-3-glucoside–catechin **3** from malvidin-3-glucoside–pyruvic acid derivative **1** and 8-vinylcatechin **2**.

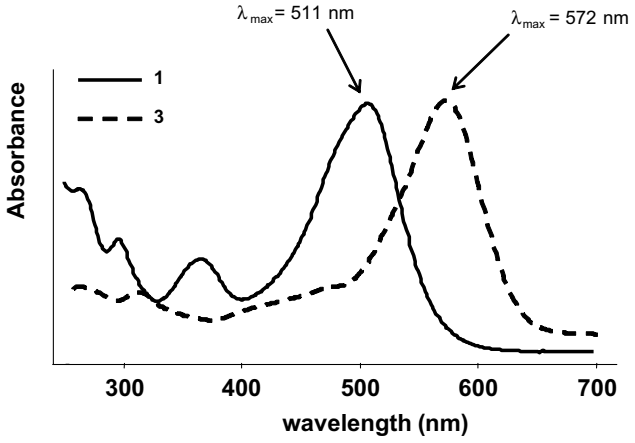


Figure 1. UV–vis spectra of the malvidin-3-glucoside–pyruvic acid derivative **1** and the vinylpyranomalvidin-3-glucoside–catechin **3**.

were assigned to two doublets with a small coupling constant ($J = 1.8$ Hz) at 6.98 and 7.06 ppm, respectively. Proton H-8A was distinguished from proton H-6A from its NOE correlation with proton H-2′B. Proton H-9 of ring D was assigned to the singlet at 8.09 ppm.

The protons of the vinyl group that revealed a clear correlation in the COSY spectrum were attributed to the two doublets located at 7.39 ppm ($J = 15.6$ Hz) and 8.45 ppm ($J = 15.6$ Hz) corresponding to H_α and H_β, respectively. The large coupling constant of these doublets suggests a *trans* stereochemistry. Moreover, two additional weak signals corresponding to two doublets were detected at 7.81 and 7.26 ppm (data not shown). These doublets revealed a smaller coupling constant ($J = 8.0$ Hz), which suggests the presence of the *cis* isomer of the portisin, although to a much lower extent.

Concerning the flavanol moiety, all the protons were also assigned. The two protons H-4αG and H-4βG were located at 3.01 and 2.63 ppm, respectively, through the characteristic AMX spin system of the pyran ring G observed in the COSY spectrum. Proton H-3G was assigned to the multiplet at 4.12 ppm from its weak (4αG) or strong (4βG) correlation with protons H-4αG and H-4βG. H-3G also correlates with H-2G, which was thus assigned to the doublet at 4.88 ppm ($J = 7.4$ Hz). This latter outcome suggests a *trans* configuration relative to H-3G, which is characteristic of (+)-catechin [12]. The position of the vinyl linkage was attributed to position 8E of the flavanol unit as proton H_α showed NOE correlations with protons H-5′F and H-6′F of ring F. These correlations would unlikely be observed if the vinyl linkage was on position 6E. In addition, a strong ^1H–^{13}C correlation (HMBC) between H_β of the vinyl linkage and C-8aE and a smaller correlation between C-8aE and H-2G, were observed thus confirming the position of the vinyl linkage on C-8E. Therefore, the only proton detected on ring E (H-6E) was attributed to the singlet at 6.87 ppm. For the protons of ring F, H-2′F was assigned to a broad doublet at 7.05 ppm, and protons H-5′F and H-6′F were attributed to the broad doublet ($J = 8.1$ Hz) at 6.97 and the double doublet ($J = 8.1$, 1.6 Hz) at 6.90 ppm, respectively.With respect to the glucosyl moiety, all the glucosyl proton signals were situated in the 3.0–3.7 ppm region, mostly masked by the methanol peak, except that of the anomeric proton, which was assigned to the doublet at 4.73 ppm ($J = 7.6$ Hz).

The assignment of the carbon resonances was possible using two-dimensional techniques (HSQC and HMBC). The correlation observed between the methoxyl proton resonances and that of the carbons at 149.3 ppm allowed their assignment to C-3′ and C-5′. C-10 was assigned at 170.8 ppm through its HMBC correlations with H_α and H_β. Carbon C-4aA was determined from its $^3J_{C,H}$ coupling with H-6. Carbon C-9D was assigned at 107.6 ppm through HSQC correlation with H-9D. The quaternary carbons C-5A, C-7A and C-8aA were assigned from their long distance correlations with protons H-8A and H-6A observed in the HMBC spectrum. Proton H_β of the vinyl group showed long distance ^1H–^{13}C correlations with C-α of the vinyl group and carbons 7E and 8aE of the flavanol moiety. Concerning the flavanol moiety, carbons C-2′, C-5′ and C-6′ of ring F were

Table 1. ^{1}H (500.13 MHz) and ^{13}C (125.77 MHz) NMR spectral data of vinylpyranomalvidin-3-glucoside–catechin **3**, in CD_3OD/TFA (98.2)

Position	δ ^{1}H (ppm); J (Hz)	δ ^{13}C
Pyranoanthocyanin moiety		
2C		160.9
3C		Nd
4C		Nd
4aA		107.7
5A		154.5
6A	6.98; d, 1.8	100.8
7A		167.4
8A	7.06; d, 1.8	100.9
8aA		153.6
9D	8.09; s	107.6
10D		170.8
1′B		121.0
2′B, 6′B	7.60; s	108.4
3′B, 5′B		149.3
4′B		142.6
3′-OMe, 5′-OMe	3.92; s	53.4
Glucose moiety		
1	4.73; d, 7.6	103.5
2	3.62	74.5
3	3.36	75.8
4	3.16	70.5
5	3.18	77.5
6a	3.73	62.0
6b	3.45	62.0
Flavanol moiety		
2G	4.88; d, 7.4	81.8
3G	4.12; m	67.6
4αG	3.01	27.0
4βG	2.63	27.0
4aE		105.9
5E		170.9
6E	6.87	102.4
7E		161.6
8E		108.2
8aE		158.1
1′F		130.1
2′F	7.05; d, 1.6	115.8
3′F		146.5
4′F		146.8
5′F	6.97; d, 8.1	116.9
6′F	6.90; dd, 8.1, 1.6	120.0
Vinyl group		
H_α	7.39; d, 15.6	115.8
H_β	8.45; d, 15.6	137.4

assigned from their direct ^{1}H–^{13}C correlations (HSQC) with H-2′F, H-5′F and H-6′F, at 115.8, 116.9 and 120.0 ppm, respectively. Carbon C-4′F assigned at 146.8 ppm was distinguished from carbon C-3′F assigned at 146.5 ppm from its long distance ^{1}H–^{13}C correlations (HMBC) with proton H-6′F, as both carbons showed long distance ^{1}H–^{13}C correlations (HMBC) with protons H-2′F and H-5′F. Finally, carbon C-1′F was assigned at 130.1 ppm from its long range ^{1}H–^{13}C correlation with H-2′F and H6′F. Carbons C-2, C-3 and C-4 of ring G were easily assigned at 81.8, 67.6 and 27.0 ppm from direct ^{1}H–^{13}C correlations in the HSQC spectrum. All the carbons of ring E could be assigned from their long range ^{1}H–^{13}C correlations with H_β, H-4G and H-6E. The quaternary carbons C-5E, C-7E and C-8aE were assigned from their long distance correlations with protons H-4G, H-6E and H_β observed in the HMBC spectrum. All the carbons of the glucosyl moiety were assigned through direct ^{1}H–^{13}C correlations in the HSQC spectrum and were situated between 62.0 and 77.5 ppm except for that at the anomeric position, which was assigned to the signal at 103.5 ppm.

Compound **3** may result from the nucleophilic attack of a 8-vinyl-catechin adduct on carbon 10 of the malvidin-3-glucoside–pyruvic acid adduct, followed by decarboxylation and oxidation. The 8-vinyl-catechin adduct maybe derived either from the cleavage of ethyl-linked catechins resulting from the acetaldehyde-induced condensation of catechins or from the dehydration of the catechin–ethanol adduct formed after reaction with acetaldehyde.[13] The extended conjugation of the π electrons in the resulting portisin structure could be the origin of its uncommon bluish colour.

Acknowledgements

The authors thank the Portugal–Spain Programme of 'Acciones integradas' for the financial support to the mobility of researchers (Ref. HP01-24 and E-23/02). This research was supported by a research project grant (POCTI/40124/QUI/2001) funded from FCT (Fundação para a Ciência e Tecnologia) from Portugal and by FEDER funding.

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