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Bisulfite addition to anthocyanins: revisited structures of colourless adducts

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Abstract

Decolourization of anthocyanins by sulfur dioxide is investigated through the reaction between sodium bisulfite and malvidin 3-*O*-glucoside. The structural elucidation of the C-4 sulfonate adducts is established by ^{1}H, ^{13}C and ^{33}S NMR spectroscopies.

Keywords : Anthocyanins ; NMR ; Sulfites ; Sulfonic acids and derivatives.

The potential use of anthocyanins as food colour additives has been restrained by their sensitivity to nucleophilic additions leading to their decolourization. This could occur in presence of sulfur dioxide as well, this one being widely used in the food industry as preservative. Even though this phenomenon has been observed for many years, the involved reaction still is not thoroughly understood. A former mechanism[1] was considering the chalcone **3**, resulting from the opening of carbinol[2,3] **2** (in equilibrium with anthocyanin **1**) to be involved, leading to a chalcone-bisulfite adduct **4**. More recent works ruled out this proposal and showed that the colourless compound, formed upon bisulfite addition either to C2 or to C4 of the anthocyanin, should be a sulfonate **5** or **6**, similar to the carbinol **2** (Fig. 1).

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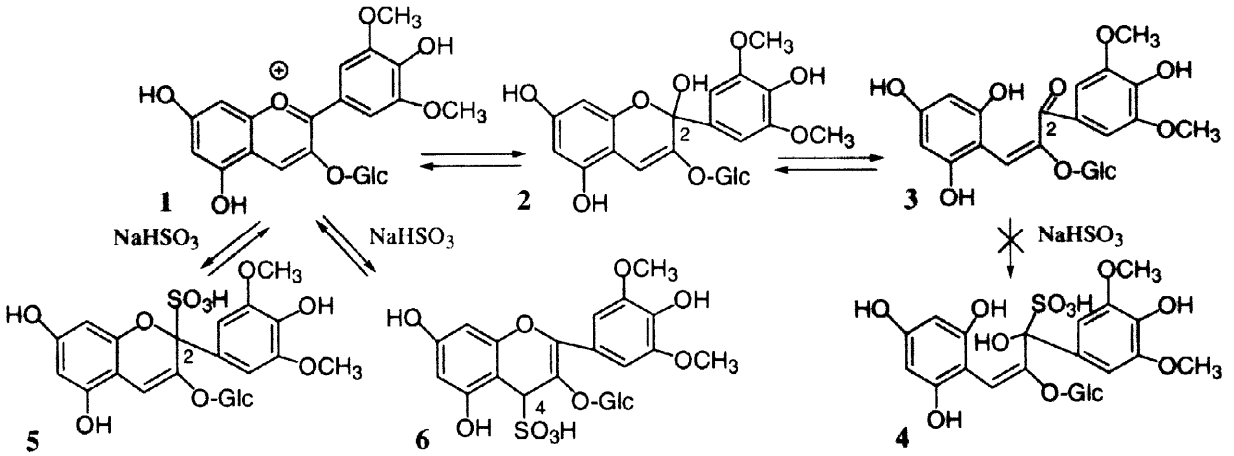

Figure 1

In this paper, we demonstrate that two bisulfite adducts are formed when malvidin 3-*O*-glucoside reacts with bisulfite, the structure of which are thoroughly proved for the first time to be **6'** and **6''** (Fig. 2). The stability of anthocyanins towards nucleophiles is then discussed. The reaction of sulfur dioxide with anthocyanins are important to be solved not only from the chemical point of view, but also due to the food industrial aspect.

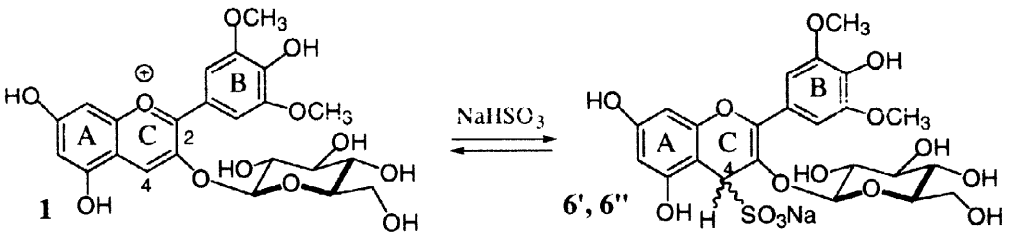

Figure 2

Results and Discussion

Sodium bisulfite is added to malvidin 3-*O*-glucoside **1**[2] until decolourized solution is observed[3]. NMR spectra of the crude mixture revealed only two subsets of resonances in a 1:1 ratio owing to the formation of diastereomers. The most apparent modifications of ^{1}H NMR spectrum of **6'** and **6''** when compared to the spectrum of **1**, are related to the loss of conjugation of the flavylium ion : a large upfield shift ($\Delta\delta$ppm = 3.3) of the H-4 resonance (δ 5.6 ppm, **6'** isomer). The most sensitive protons to the newly formed stereocenter at C-4 in terms of chemical shift are the closest situated ones *i.e.* the H-1''. Due to the chiral property of this anomeric proton, a large chemical shift difference between the two diastereomers is

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[2] Malvidin 3-*O*-glucoside **1** (from grapes). UV λ_{max}^{MeOH} nm 537. FAB positive MS (glycerol) : m/z 493. Assignments made from 2D heteronuclear measurements : ^{1}H NMR (500 MHz in CD_3OD-TFA 5%) δ ppm, 3.43 (H-4"), 3.55 (2H ; H-3" and H-5"), 3.62 (H-2"), 3.71 (H-6"a), 3.91 (H-6"b), 3.94 (OMe), 5.3 (H-1"), 6.58 (H-6), 6.83 (H-8), 7.8 (2H ; H-2' and H-6'), 8.9 (H-4). ^{13}C NMR (125 MHz) δ ppm 57.6 (OMe), 62.7 (C-6"), 71.7 (C-4"), 75.5 (C-2"), 78.8 (C-5"), 79.3 (C-3"), 95.9 (C-8), 104.0 (C-6), 104.5 (C-1"), 111,2 (C-2' and C-6'), 114.2 (C-4a), 120.3 (C-1'), 137.5 (C-4), 146.2 (C-3), 147.3 (C-4'), 150.6 (C-3' and C-5'), 158.6 (C-8a), 160.3 (C-5), 164.1 (C-2), 172.0 (C-7).

[3] Adducts are prepared from a solution of **1** (50 mg, 0.095 mmole in 0.5 ml D_2O/DCl 5%) in an NMR tube to avoid their denaturation. Sodium bisulfite is added until absorption at λ_{nm}^{MeOH} 537 becomes very weak (416 mg, 4 mmoles).

observed (H-1'' in **6'** = 5.3 ; H-1'' in **6''** = 4.8).

The J-modulated[4] ^{13}C NMR spectrum also speaks in favour of such a modification with the C-4 methine carbon at δ 56.9 ppm (**6'** isomer), characteristic of a benzylic carbon bearing an heteroatom. These assignments are also confirmed by the HMQC correlation (δH 5.6/δC 56.9, **6'** isomer).

The addition took place on the C-4 of the flavylium, to form bisulfite-adduct (either through its oxygen (sulfinate) or its sulfur atom (sulfonate). In order to discriminate between the two possibilities, the ^{33}S NMR spectrum is measured using aring sequence to avoid acoustic ringing effects[5]. On the basis of the chemical shift (δ - 40 ppm relative to sulfolane) and of the narrow linewidth ($W_{1/2}$ = 40 Hz), the observed signal can only be due to a sulfonate derivative[6,7]. Indeed, the linewidth of ^{33}S signal is a powerful structural information tool as it is related to sulfur environment : for symmetric compounds (sulfonates) it is narrow (tenth of Hz), but for unsymmetrical ones (sulfinates) it reaches KHz. The measured values, in our case, clearly prove the sulfonate nature of the adducts **6'** and **6''**.

Complete assignments of the ^{1}H and ^{13}C resonances are made using heteronuclear 2D NMR spectroscopy. HMQC[8] and HMBC[9] correlations indicate that the A and B rings as well as the glucosyl moiety keep closely identical to those of malvidin-3-*O*-glucoside **1**, while the C-ring is modified.

The most relevant long-range correlations (dotted lines, in Fig. 3) are those of H-2'/H-6' (7.45 ppm, **6'** isomer) and H-4 with carbon at 146.4 ppm allowing us to assign C-2 carbon. Other main correlations are summarized on Fig. 3 and the deduced chemical shifts are reported for each compound[4].

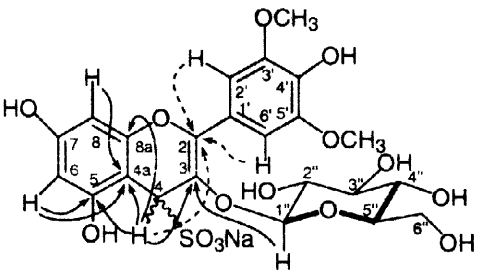

Figure 3 : Main HMBC correlations

The combined use of ^{1}H-^{13}C 2D NMR and ^{33}S NMR spectroscopies allowed the unambiguous assignments of protons and carbon atoms of the two diastereomers resulting from bisulfite nucleophilic addition either on the *Si* face or on the *Re* face at the sp2 C-4 center of the flavylium **1**. As these isomers are formed in equal amounts (from NMR), it is assumed that bisulfite adds equally on both faces without any interaction with the chiral glucosyl residue.

The NMR data confirmed that the competitive bonding between water and bisulfite is in favour of the latter, this being valid in acidic media as it has been previously demonstrated by BROUILLARD[10] in term of kinetic and thermodynamic studies.

These only formed structures, thus securely determined, definitely confirm the previous proposals by BROUILLARD[10] and JURD[6] but reject the one from GLORIES[11] which concluded to a C-2 adduct. In a similar case, nucleophilic addition of methoxide ion on

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[4] Compound **6'**: ^{1}H NMR (500 MHz in CD_3OD-TFA 5%) δ ppm, 3.54 (H-3"), 3.62 (H-2"), 3.68 (H-4"), 3.77 (H-5"), 3.85 (H-6"a), 3.93 (H-6"b), 4.05 (OMe), 5.3 (H-1"), 5.6 (H-4), 6.52 (H-6), 6.55 (H-8), 7.45 (2H ; H-2' and H-6'). ^{13}C NMR (125 MHz) δ ppm 56.9 (C-4), 59.7 (OMe), 63.3 (C-6"), 72.1 (C-4"), 75.9 (C-2"), 78.2 (C-3"), 78.5 (C-5"), 98.7 (C-8), 102.0 (C-4a), 102.3 (C-1") 102.4 (C-6), 108.9 (C-2' and C-6'), 126.1 (C-1'), 131.4 (C-3), 137.6 (C-4'), 146.4 (C-2), 149.9 (C-3' and C-5'), 156.6 (C-8a), 157.1 (C-5), 159.6 (C-7). Compound **6''**: ^{1}H NMR δ ppm, 3.56 (H-3"), 3.6 (2H ; H-4" and H-5"), 3.65 (H-2"), 3.78 (H-6"a), 3.93 (H-6"b), 4.1 (OMe), 4.8 (H-1"), 5.55 (H-4), 6.55 (H-6), 6.62 (H-8), 7.35 (2H ; H-2' and H-6'). ^{13}C NMR δ ppm 59.7 (OMe), 61.2 (C-4), 63.4 (C-6"), 72.2 (C-4"), 76.2 (C-2"), 78.3 (C-3"), 78.6 (C-5"), 98.7 (C-8), 106.8 (C-1"), 102.4 (C-6), 103.0 (C-4a), 109.4 (C-2' and C-6'), 125.4 (C-1'), 133.0 (C-3), 138.5 (C-4'), 147.5 (C-2), 150.1 (C-3' and C-5'), 156.8 (C-8a), 157.4 (C-5), 159.8 (C-7).

diphenylpyrylium has been reported[12] to take place on the more accessible C-4 position because of its lower steric hindrance with respect to the C-2 position. This argument could also apply in our case. In other experiments, butanedione, for example, reacted only once with bisulfite, while glyoxal did twice. Thus, it is clear that capacity of bisulfite to add to electrophiles depends on their steric hindrance. It is conceivable that to prevent such an addition to flavylium, it should be sufficient to introduce a bulky group around the C-4 position, as it has been observed on flavylium derivatives[13].

The malvidin 3-*O*-glucoside **1** is sensitive to bisulfite decolourization by forming 4α and 4β-sulfonate adducts **6'** and **6''** but this reaction is clearly a reversible one and, in the usual conditions (when the medium does not content bisulfite excess, as it must be in the case of wine), the equilibrium is almost quantitatively in favour of the flavylium cation.

Conclusion

The adducts **6'** and **6''** from the reaction of bisulfite on malvidin 3-*O*-glucoside **1** have been determined for the first time by ^{1}H, ^{13}C and ^{33}S NMR spectroscopies. The ^{33}S NMR is necessary to demonstrate the sulfonate nature of the bisulfite-adducts, whereas ^{1}H and ^{13}C two-dimensional NMR are indicative of the addition to the C-4 carbon.

Sulfur dioxide reactivity, as an electrophile, as a nucleophile or as an acid, when added to foods, is under investigation in our laboratory, by the use of ^{33}S NMR that we proved to be possible, even at the (IV) level of oxidation.

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