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Analogs of anthocyanins with a 3′,4′-dihydroxy substitution: Synthesis and investigation of their acid–base, hydration, metal binding and hydrogen-donating properties in aqueous solution

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ABSTRACT

Glycosides of hydroxylated flavylium ions are proposed as pertinent analogs of anthocyanins, a major class of polyphenolic plant pigments. Anthocyanins with a 3′,4′-dihydroxy substitution on the B-ring (catechol nucleus) are especially important for their metal chelating and electron-donating (antioxidant) capacities. In this work, an efficient chemical synthesis of 3′,4′-dihydroxy-7-*O*-β-D-glucopyranosyloxy-flavylium chloride and its aglycone is reported. Then, the ability of the two pigments to undergo proton transfer (formation of colored quinonoid bases) and add water (formation of a colorless chalcone) is investigated: at equilibrium the colored quinonoid bases (kinetic products) are present in very minor concentrations (<10% of the total pigment concentration) compared to the colorless chalcone (thermodynamic product). The glucopyranosyloxyflavylium ion appears significantly less acidic than the aglycone. The thermodynamics of the overall sequence of flavylium – chalcone conversion is not affected by the β-D-glucosyl moiety while the kinetics appears slower by a factor *ca.* 8. Although the glucopyranosyloxyflavylium ion and its aglycone display similar affinities for Al^{3+}, the Al^{3+}-glucoside complex is more stable than the Al^{3+}-aglycone complex due to the higher sensitivity of the latter to water addition and conversion into the corresponding chalcone. Finally, the glucopyranosyloxyflavylium ion and its aglycone are compared for their ability to reduce the 1,1-diphenyl-2-picrylhydrazyl radical in a mildly acidic water/MeOH (1:1) mixture as a first evaluation of their antioxidant activity. Glycosidation at C_7-OH results in a lower rate constant of first electron transfer to DPPH and a lower stoichiometry (total number of 1,1-diphenyl-2-picrylhydrazyl radicals reduced per pigment molecule).

Anthocyanins are difficult to extract from plants in substantial amount. However, the analogs investigated in this work are of easy access by chemical synthesis and express the physico-chemical properties typical of anthocyanins. They can thus be regarded as valuable models for investigating the coloring, metal-binding and antioxidant properties of these important natural pigments.

1. Introduction

Polyphenols with a 1,2-dihydroxybenzene (catechol) group are common in plants and in our diet. This is for instance the case of caffeic acid (3,4-dihydroxycinnamic acid) and 3′,4′-dihydroxy-flavonoids such as quercetin, (epi)catechin, cyanidin and their derivatives (O-glycosides, esters, oligomers) [1,2]. Those polyphenols are of special interest for their ability to bind metal ions and readily transfer electrons or H-atoms to radicals. As such, they are typically strong in vitro antioxidants. Although the relatively poor bioavailability and extensive metabolism of polyphenols in humans [3] severely restrict the biological significance of in vitro antioxidant tests, it is reasonable to assume that polyphenols with a catechol group may be very important antioxidants in plant and food, and possibly in the digestive tract [4,5].

Anthocyanins are naturally occurring glycosides of flavylium (2-phenyl-1-benzopyrylium) ions substituted by hydroxyl and methoxyl groups [6]. As most polyphenols, they are mainly stored in the mildly acidic aqueous environment of vacuoles within plant cells. Anthocyanins constitute one of the major classes of plant pigments, typically responsible for a wide variety of red to blue colors [7]. One important mechanism of color stabilization and variation is metal – anthocyanin binding, a phenomenon restricted to 3′,4′-dihydroxyflavylium ions. In particular, most blue colors

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found in Nature are complexes of anthocyanins with magnesium, aluminum or iron ions. On the other hand, anthocyanins are also one of the most ubiquitous polyphenol classes in foods, e.g. berries and their products (juices, jams, red wine), red cabbage, red onion and eggplant [2]. As such, anthocyanins may contribute to the protective effects of diets rich in plant products [8]. However, investigating the chemical properties of anthocyanins in line with their activity in plant, food and in humans is somewhat impeded by their difficult extraction and purification from plants and their limited access from commercial sources. Hence glycosides of hydroxylated flavylium ions can be proposed as pertinent anthocyanin analogs. In this work, an efficient chemical synthesis of 3′,4′-dihydroxy-7-*O*-β-D-glucopyranosyloxyflavylium chloride and its aglycone is reported as well as the ability of the two pigments to undergo proton transfer, add water with subsequent conversion into chalcones, bind Al^{3+} and deliver electrons to the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical.

2. Experimental

2.1. Materials and instruments

All starting materials were obtained from commercial suppliers and were used without purification. Purifications were performed by column chromatography on Merck Si60 silica gel (40–63 μm) and by elution on Varian bond elut C18 silica gel cartridges.

^{1}H and ^{13}C NMR spectra were recorded on an Advance DPX300 Bruker apparatus at 300.13 MHz (^{1}H) or 75.46 MHz (^{13}C). Chemical shifts (δ) in ppm relative to tetramethylsilane, ^{1}H–^{1}H coupling constants (J) in Hz. High-resolution mass spectrometry (HRMS) analyses were carried out on Qstar Elite instrument (Applied Biosystems SCIEX). Mass detection was performed in the positive electrospray ionization mode. HPLC analyses were performed on a Waters HPLC system consisting of a 600E pump, a 717 autosampler, a 2996 photodiode array detector, an in-line AF degasser and a Millenium workstation. A LichroCart 250-4 Lichrospher 100 RP18e column (250 × 4.6 mm, 5 μm particle size) was used for chromatographic separations at 25 °C. The solvent system was a gradient of A (5% HCO_2H in MeCN/H_2O 1/1) and B (5% HCO_2H in H_2O) with 10% A at 0 min and 100% A at 60 min (flow rate = 1 mL min^{-1}). UV–Vis absorption spectra were recorded on an Agilent 8453 diode array spectrometer equipped with a magnetically stirred quartz cell (optical path length = 1 cm). The temperature in the cell was controlled by means of a water-thermostated bath at 25 ± 0.1 °C.

2.2. Chemical syntheses

2.2.1. 3,4-Dihydroxyacetophenone

A mixture of activated zinc powder (5 g, 76 mmol), ω-chloro-3,4-dihydroxyacetophenone (5 g, 27 mmol), THF (120 mL) and acetic acid (30 mL) was vigorously stirred for 2 days at room temperature. After filtration and concentration under reduced pressure, EtOAc (100 mL) was added. The organic layer was washed with water (3 × 100 mL), dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography (SiO_2, cHex/EtOAc, 1:1 v/v) to give compound 3,4-dihydroxyacetophenone as a white amorphous powder (3.7 g). Yield 90%. ^{1}H NMR [$CDCl_3$]: δ = 2.53 (s, 3H, $COCH_3$), 5.99 (1H, s, OH), 6.19 (1H, s, OH), 6.96 (1H, d, J = 8.3, H_5), 7.55 (1H, dd, J = 2.0 and 8.3, H_6), 7.67 (1H, d, J = 2.0, H_2). ^{13}C NMR [$CDCl_3$]: δ = 24.9 (CH_3), 114.4, 114.6 (C_1, C_5), 122.2 (C_2), 129.2 (C_6), 145.0 (C_3), 150.9 (C_4), 198.4 (C=O). HPLC-UV/Vis t_R = 14.2 min, λ_{max} = 276 nm.

2.2.2. 3′,4′,7-Trihydroxyflavylium chloride (**P1**)

A solution of equimolar amounts (4 mmol) of 2,4-dihydroxybenzaldehyde and 3,4-dihydroxyacetophenone in distilled EtOAc (10 mL) was cooled to 0 °C. Gaseous HCl (generated by action of 98% H_2SO_4 on solid NaCl) was gently bubbled through the solution for 90 min. The mixture was kept at 4 °C for 3 days, then filtered. More precipitate was collected after evaporation of the filtrate and addition of diethyl ether (Et_2O). After precipitation in EtOAc, **P1** was obtained as a red powder (0.651 g, yield 56%). The purity of **P1** was carefully checked by reversed-phase HPLC. ^{1}H NMR [0.2 M TFA-d in CD_3OD]: δ = 7.08 (1H, d, J = 8.8, $H_{5'}$), 7.40 (1H, dd, J = 2.2 and 8.8, H_6), 7.46 (1H, d, J = 2.2, H_8), 7.84 (1H, d, J = 2.2, $H_{2'}$), 8.00 (1H, dd, J = 2.2 and 8.8, $H_{6'}$), 8.11 (1H, d, J = 8.8, H_5), 8.24 (1H, d, J = 8.8, H_3), 8.99 (1H, d, J = 8.8, H_4). ^{13}C NMR [0.2 M TFA-d in CD_3OD]: δ = 103.0 (C_8), 112.9 (C_3), 115.7 ($C_{2'}$), 117.3 ($C_{5'}$), 119.0 (C_{10}), 121.0 ($C_{1'}$), 121.5 (C_6), 125.7 ($C_{6'}$), 133.1 (C_5), 147.8 ($C_{3'}$), 153.1 (C_4), 156.7 ($C_{4'}$), 159.4 (C_9), 169.3 (C_7), 173.1 (C_2). HRMS m/z = 255.0652 (M^+, 255.0652 calculated for $C_{15}H_{11}O_4^+$). UV/Vis (0.13 M aqueous HCl): ε(470 nm) = 38,400 M^{-1} cm^{-1}. HPLC-UV/Vis t_R = 24.1 min, λ_{max} = 472 nm.

2.2.3. 4-(2′,3′,4′,6′-Tetra-O-acetyl-β-D-glucopyranosyloxy)-2-hydroxybenzaldehyde

A solution of tetra-*O*-acetyl-α-D-glucopyranosylbromide (9.25 g, 1.5 equiv.) in CH_2Cl_2 (25 mL) was added to a solution of 2,4-dihydroxybenzaldehyde (2.07 g, 15 mmol) and tris(2-(2-methoxyethoxy)ethyl)amine (7.20 mL, 1.5 equiv.) in 1 M $NaHCO_3$/1 M KCl (25 mL, 1/1, v/v). The mixture was refluxed for 48 h. After addition of H_2O (100 mL) and extraction with CH_2Cl_2 (3 × 100 mL), the combined organic phases were successively washed with 1 M HCl (2 × 100 mL) and H_2O (2 × 100 mL), dried over Na_2SO_4 and concentrated. The residue was purified on silica gel (eluent EtOAc/cyclohexane (3/7, v/v)) to afford the target compound as a white powder (5.62 g, yield 80%). ^{1}H NMR [$CDCl_3$]: δ = 2.08 (12H, s, 4 CH_3 of Ac groups), 3.95 (1H, m, $H_{5'}$), 4.17–4.33 (2H, 2dd, J = 12.4 and 5.8, J = 12.4 and 2.3, $H_{6'}$), 5.14–5.32 (4H, m, $H_{1'}$, $H_{2'}$, $H_{3'}$, $H_{4'}$), 6.54 (1H, s, H_3), 6.59 (1H, d, J = 8.5, H_5), 7.47 (1H, d, J = 8.5, H_6). ^{13}C NMR [$CDCl_3$]: δ = 20.6 (4 CH_3 of Ac groups), 61.8 ($C_{6'}$), 68.1 ($C_{4'}$), 70.8 ($C_{2'}$), 72.3 ($C_{5'}$), 72.5 ($C_{3'}$), 97.6 ($C_{1'}$), 103.5 (C_3), 109.6 (C_5), 116.6 (C_6), 135.4 (C_1), 163.1 (C_2), 164.0 (C_4), 169.2–170.6 (4 C=O of Ac groups), 194.9 (CHO).

2.2.4. 3′,4′-Dihydroxy-7-O-β-D-glucopyranosyloxyflavylium chloride (**P2**)

Equimolar amounts (1 mmol) of 3,4-dihydroxyacetophenone and 4-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosyloxy)-2-hydroxybenzaldehyde were dissolved in dry EtOAc (10 mL) and cooled to 0 °C. Gaseous HCl was gently bubbled through the solution under stirring during 60 min. The deep-red solution was then allowed to stay at −18 °C for 6 days and filtered. Et_2O was added to the filtrate to ensure complete precipitation. The solid was dissolved in MeOH (20 mL) under Ar and a solution of MeONa in MeOH was added until pH 9 (wet pH paper). After stirring for 1.5 h at room temperature, 1 M HCl was added until pH 1 (wet pH paper). The mixture was kept at 4 °C for 12 h, then concentrated under reduced pressure. The residue was dissolved in 0.01 M HCl (2 mL) and loaded on a C18 cartridge. After elution with 100 mL of 0.01 M HCl to remove contaminating NaCl, **P2** was eluted with 70 mL of 0.2 M HCl in MeOH. After evaporation of solvent under reduced pressure, **P2** was obtained as a red powder (0.34 g, yield 75%). The purity of **P2** was carefully checked by reversed-phase HPLC. ^{1}H NMR [0.2 M TFA-d in CD_3OD]: δ = 3.47 (1H, *t*, J = 9.2, $H_{4''}$), 3.55–3.61 (2H, m, $H_{3''}$, $H_{2''}$), 3.69–3.79 (2H, m, $H_{5''}$, $H_{6''}$), 3.99 (1H, m, $H_{6''}$), 5.37 (1H, d, J = 6.7, $H_{1''}$), 7.11 (1H, d, J = 8.8, $H_{5'}$), 7.63 (1H, dd, J = 2.2 and 8.9, H_6), 7.91 (1H, d, J = 2.2, H_8), 7.95 (1H, d, J = 2.2,