

Synthesis of Flavanol-4-ol and its Spectroscopic Properties in Aqueous Solution

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The polyhydroxybenzene derivatives can act as anti-oxidants in living organisms and for this reason considerable effort has been devoted to them.^{1,2} Particularly, the plant-derived polyphenols have received great attention due to their various biological activities, *i.e.*, anticarcinogenic, antiatherosclerotic, antimicrobial, and antioxidant properties.¹⁻⁸ Polyphenolic substances are commonly referring to the subgroup flavanols that belong to the flavonoid family. They can be easily found in green tea, red wine and numerous fruits and vegetables.⁹⁻¹¹ In addition many of the phenolic phytochemical compounds can also be related to the presence of flavanol moieties in their structure.

One of the such substances is catechin (5,7,3',4'-tetrahydroxyflavan-3-ol) that is the major constituent of green tea and also one of the flavanol compounds. This substance is known to repair vitamin E as well as to be very efficient scavengers for biologically damaging oxy radicals, such as the superoxide radical and singlet oxygen.^{12,13} However, catechin is oxidized in water easily to form a yellow solution where the color of the solution varies with time.¹⁰ This is because the catechol moiety on the B-ring in catechin is oxidized in an aqueous solution.^{10,14} Recently, we confirmed that the oxidation in a basic solution is much faster than in the neutral catechin solution.¹⁴ Besides, the oxidation depended strongly on the pH values of the solution where higher pH values led to faster oxidation rate of catechin. However, flavan-4-ol (2-Phenylchroman-4-ol) is not contained catechol moiety in the molecule.

In this paper, we have synthesized flavan-4-ol (2-phenylchroman-4-ol) and the spectroscopic properties of the substance were evaluated to explain its oxidation in the aqueous solution.

Experimental Section

1-(2-Hydroxyphenyl)ethanon, benzaldehyde were purchased from Sigma Chemical Co. (St. Louis, U.S.A.) and used without further purification. The other chemicals were reagent grade and used as received. Flavan-4-ol (2-phenylchroman-4-ol) was synthesized by starting from 1-(2-hydroxyphenyl)ethanon with benzaldehyde in ethanol. Flavan-4-ol synthesized was very slightly soluble in water. Therefore, we first prepared the stock solution of flavan-4-ol in methanol. To prepare the aqueous flavan-4-ol, an aliquot of flavan-4-ol stock solution was transferred to a flask and then vaporized

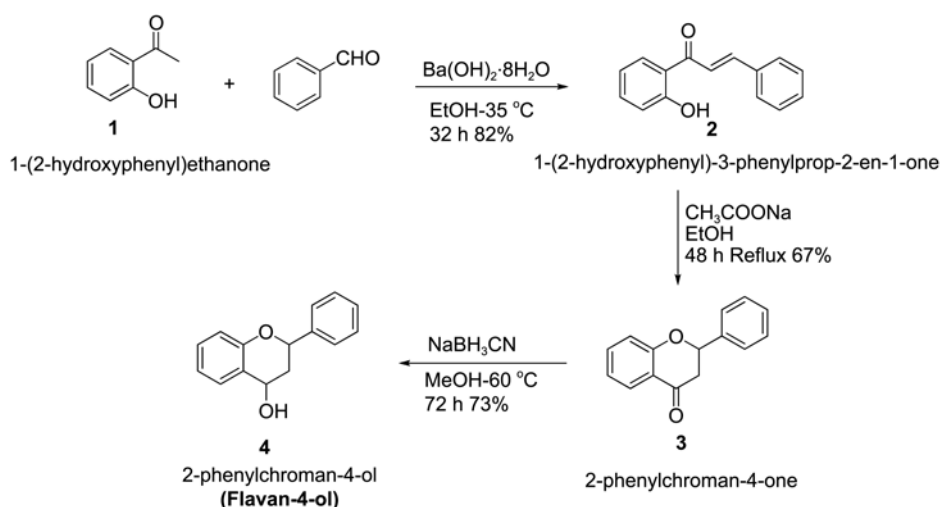
by bubbling high purity argon (99.999%) through. The compound was then dissolved in water using double distilled water, which was obtained by passing distilled water through a Barnstead (U.S.A.) Nanopure II deionization system. The pH of the solution was adjusted by adding HClO₄, NaOH or ammonium chloride buffer solutions which were prepared from air-free distilled water. These buffer solutions did not absorb any UV light with wavelength longer than 240 nm and therefore would not overlap with the characteristic peaks of flavan-4-ol.

The UV/vis absorption spectra of the solutions were then taken by a UV/vis spectrophotometer (Uvikon, model 943, Italy) at room temperature. The steady-state fluorescence emission spectra were obtained on a Varian Cary Eclipse spectrofluorometer with 5 nm slits at room temperature after eliminating the oxygen from the solution by means of bubbling high purity argon (99.999%) through. To improve the accuracy, each sample was scanned 10 times and the average of these measurements was recorded as the emission spectrum.

Results and Discussion

Flavan-4-ol is the basic unit for the flavonoids and the flavanols which can be commonly found in plants such as abacopteris penangiana species and sorghum. It could be isolated from the rhizomes of abacopteris penangiana in the form of flavan-4-ol glycosides, (2R,4S)-6,8-dimethyl-7-hydroxy-4'-methoxy-4,2"-oxidoflavan-5-O-β-d-6"-O-acetylglucopyranoside (1) and (2R,4S)-5,7-O-β-d-di-glucopyranosyloxy-4'-methoxy-6,8-dimethyl-4,2"-oxidoflavane.^{15,16} Since the extracted products are combined with glycosides, we synthesized flavan-4-ol (2-phenylchroman-4-ol) starting from 1-(2-hydroxyphenyl)ethanon with benzaldehyde in ethanol as presented in Scheme 1. The condensation with 1-(2-hydroxyphenyl)ethanon and benzaldehyde in the presence of Ba(OH)₂·8H₂O under EtOH resulted in the α,β-unsaturated ketone 2 with 82% yield. The cyclization of 2 was conducted with sodium acetate under reflux condition in ethanol. We were not satisfied with the results from the Yang's method which employs phosphoric acid in ethanol.¹⁷ In addition the reduction of ketone 3 with NaBH₃CN in methanol at 60 °C for 72 h gave the corresponding alcohol 4 in 73% yield.

Preparation of 1-(2-Hydroxyphenyl)-3-phenylprop-2-



Scheme 1

en-1-one: 1-(2-hydroxyphenyl) ethanone (5.0 g, 36.6 mmol) was dissolved in ethanol (25 mL) followed by the addition of benzaldehyde (3.88 g, 36.6 mmol) and barium hydroxide octahydrate. The reaction mixture was stirred at 35 °C for 32 hours before poured into water and then the pH value of which was adjusted to 7.0. After the addition of ethyl acetate, the organic layer was separated and dried over MgSO_4 , and then evaporated under reduced pressure. The crude residue was purified by column chromatography (hexane:EtOAc/10:1) to afford a pale yellow solid with a yield of 6.75 g (82%, 30.14 mmol).

$^1\text{H NMR}$ (300 MHz, CDCl_3): δ 12.81 (s, 1H), 7.95-7.90 (m, 2H), 7.68-7.63 (m, 3H), 7.53-7.43 (m, 4H), 7.04-6.92 (m, 2H).

Preparation of 2-Phenylchroman-4-one. 1-(2-Hydroxyphenyl)-3-phenylprop-2-en-1-one (3.00 g, 13.4 mmol) was dissolved in ethanol (15 mL) with the addition of sodium acetate (1.32 g, 16.1 mmol). The reaction mixture was refluxed for 48 hours before poured into water, and then ethyl acetate was added to the mixture followed by stirring. The organic layer was separated and dried over MgSO_4 to obtain the crude product. This crude residue was then purified by column chromatography (hexane:EtOAc, 10:1) to afford a white solid with a yield of 2.01 g (67%, 9.00 mmol). $^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.94 (dd, $J = 3.6, 1.8$ Hz, 1H), 7.54-7.36 (m, 6H), 7.10-7.03 (m, 2H), 5.49 (dd, $J = 10.2, 3.0$, 1H), 3.10 (m, 1H), 2.90 (m, 1H).

Preparation of 2-Phenylchroman-4-ol: 2-Phenylchroman-4-one (1.00 g, 4.46 mmol) was dissolved in methanol (10 mL) with the addition of sodium cyanoborohydride (1.32 g, 44.6 mmol). The reaction mixture was stirred at 60 °C for 72 hours before poured in water followed by subsequent addition of brine solution and ethyl acetate. The resulting solution was stirred and its organic layer was separated and dried over MgSO_4 . After evaporation, the crude product was obtained which was then purified by column chromatography (hexane:EtOAc/4:1) to afford a white solid product with a yield of 0.74 g (73%, 3.30 mmol).

$^1\text{H NMR}$ (300 MHz, CDCl_3) δ 7.54-7.31 (m, 6H), 7.25-7.18 (m, 1H), 7.02-7.68 (m, 2H), 5.18 (dd, $J = 2.1, 1.8$, 1H),

5.15 (br m, 1H), 2.53 (m, 1H), 2.20-2.01 (m, 1H), 1.77 (d, $J = 8.7$ Hz, 1H); $^{13}\text{C NMR}$ (75 MHz, CDCl_3) δ 154.5, 140.5, 129.2, 128.7, 128.2, 127.0, 126.1, 125.7, 121.0, 116.7, 76.8, 65.8, 40.1.

Spectroscopic Properties of Flavan-4-ol. Flavan-4-ol is only slightly soluble in water but aqueous flavan-4-ol can be oxidized in the presence of oxygen. Therefore to investigate this potential oxidation process, the aqueous solution of flavan-4-ol was prepared using methanolic stock solution as mentioned in the experimental session. To characterize the aqueous flavan-4-ol solution, an aliquot of flavan-4-ol was dispersed in water and left in dark at room temperature for 5 days. The resulting solution did not display any remarkable difference since its UV-vis absorption spectra stayed the same as time passes after adding flavan-4-ol to water. Consequently this indicates that flavan-4-ol is not affected by the presence of oxygen in the pH neutral water.

The UV-vis absorption spectrum of 67 μM flavan-4-ol reveals the absorption maximum at 275 nm in water. The UV-visible absorption properties of the acidic flavan-4-ol

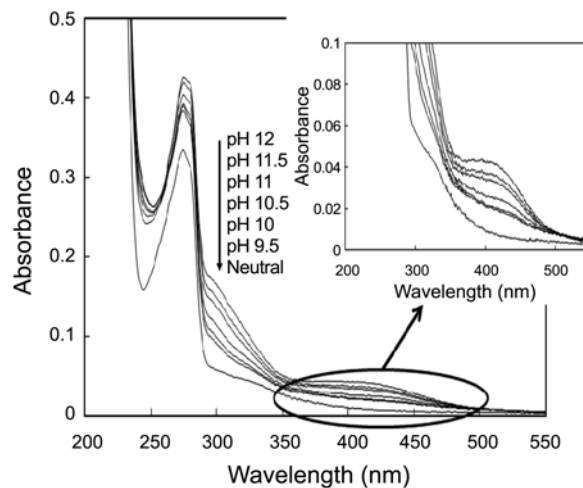
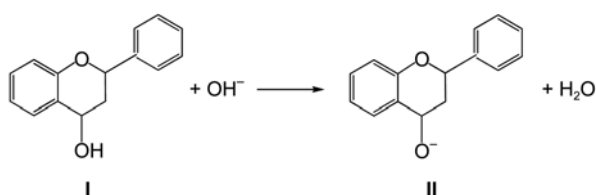


Figure 1. UV-vis absorption spectra of 67 μM flavan-4-ol in various basic aqueous buffer solutions.

solution are quite similar to those of the pH neutral water. When the pH values of the aqueous flavan-4-ol solutions increased, only the absorption spectra of the basic ones were affected, as shown in Figure 1. The absorbance intensity at 300 nm increased, and new peaks appeared at the wavelength of 410 nm.

Overall, the higher the pH value of the solution, the stronger the absorption appears to be. This suggests that the hydroxide ion generated in the basic aqueous solution affects the molecular structure of flavan-4-ol. A possible reaction mechanism is the dissociation of flavan-4-ol as following.



The dissociation of flavan-4-ol is facilitated by the increase of the pH value in the solution. However, we could not find an isosbestic point on the absorption spectra and this might be attributed to the fact that flavan-4-ol molecules have not reached the equilibrium state between structure I and II under our experimental conditions.

Since phenoxide ion is altered due to its rearrangement to ketone form,¹⁸ we examined the stability for the structure II using fluorescence spectroscopy. Substances that display significant fluorescence generally possess delocalized electrons that are present in conjugated double bonds.¹⁹ The steady-state fluorescence emission spectra of 67 μM flavan-4-ol were, therefore, measured in the aqueous basic buffer solution. Their emission spectra were obtained in the range of 290 nm to 370 nm with a maximum at 303 nm and also in the range of 370 nm to 580 nm with a maximum at 411 nm. As shown in Figure 2, the fluorescence intensity of the solution hardly changed with increasing pH values. This finding illustrated that the anionic form of flavan-4-ol did not undergo any further transformation. These results thus

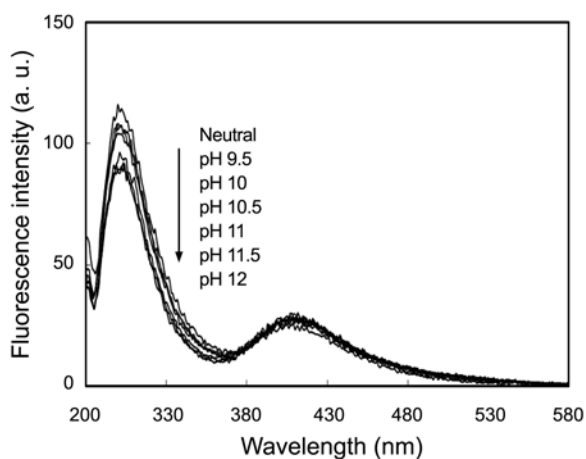


Figure 2. Fluorescence spectra (excitation at 280 nm) of 67 μM flavan-4-ol in various basic aqueous buffer solutions.

allow us to predict that the anionic form of flavan-4-ol (structure II) is very stable under our experimental conditions.

In summary, we synthesized flavan-4-ol (2-phenylchroman-4-ol) with good yield from 1-(2-hydroxyphenyl)ethanon with benzaldehyde in ethanol. Flavan-4-ol is not affected by the presence of oxygen in the pH neutral water. The UV-vis absorption spectrum of 67 μM flavan-4-ol reveals a maximum at 275 nm in water which remained the same as time passes after adding flavan-4-ol to water. However, their absorption spectra depended strongly on the pH values in the basic solutions. The higher the pH value of the solution, the stronger the absorption appears to be. The steady-state fluorescence emission spectra of 67 μM flavan-4-ol did not display any other remarkable difference with increasing pH values of the solution. This finding indicates that the anionic form of flavan-4-ol is very stable under our experimental conditions.

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