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UV- Vis Shift reagent study on 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-8-((3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-2-pyranyl)oxy)-4H-4-chromenone

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Abstract

We have studied the characteristic shift in absorption using versatile UV-Visible shift reagents for the compound **I** namely, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-8-((3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-2-pyranyl)oxy)-4H-4 chromenone which was discovered earlier in our laboratory from the plant extract of *Hibiscus calyphyllus* flowers.

Key Words: Novel Flavonol, UV-Vis Spectra, Shift reagents, Absorption studies

Introduction

UV – Vis shift reagents play a vital role in the characterization of substitution pattern of flavonoid nucleus and the preparation of these reagents are easy, rapid and unswerving.¹ The UV spectra of flavonols usually exhibit two major absorption peaks in the region 240-400 nm.²⁻³ These two peaks are commonly referred to as Band I (usually 300-380 nm), and Band II (usually 240-280 nm). Band I and II are considered to be associated with absorptions due to the B-ring cinnamoyl system and A-ring benzoyl system respectively.⁴⁻⁶ In view of that, the substitution pattern of compound **I**, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-8-((3,4,5-trihydroxy-6-(hydroxyl methyl)tetrahydro-2H-2-pyranyl)oxy)-4H-4-chromenone (**Figure 1**) can be identified on the basis of shift in absorption maximum caused by addition of different shift reagents.

Materials and Methods

UV spectra were recorded on HITACHI UH5300 UV-Vis spectrophotometer by adding shift reagents of Sodium acetate, Aluminium chloride, AlCl₃ + HCl mixture, Sodium methoxide, Sodium ethoxide, Boric acid and Acetic acid. Compound **I**, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-8-((3,4,5-trihydroxy-6-(hydroxylmethyl)tetrahydro-2H-2-pyranyl)oxy)-4H-4-chromenone was previously isolated from our laboratory. About 0.2 mg of compound **I** is separately dissolved in 20 mL of ethanol and methanol and used as stock solution for recording UV-Vis spectra. Spectroscopic grade of methanol / ethanol without added reagent was used as reference.

Experimental Section

Preparation of shift reagents

(1) Ethanol

The alcoholic stock solution (3.5 mL) is taken in a cuvette and analyzed for UV-Vis spectra.

(2) Ethanol + NaOAc

Preparation of Sodium acetate reagent

About 0.82 g of sodium acetate (0.01 N) is dissolved in small amount of water and the solution is made up into 100 mL SMF.

A drop of the sodium acetate reagent is mixed well with the ethanolic stock solution and was analysed UV-Vis spectra.

(3) Ethanol + $AlCl_3$

Preparation of Aluminium chloride reagent

Anhydrous aluminium chloride (3.3 g) was cautiously added to 50 mL ethanol and it dissolved after 24 h afforded 5% solution, and the reagent was used as a shift reagent.

A drop of the Aluminium chloride reagent is added to the ethanolic stock solution and recorded UV-Vis spectra.

(4) Ethanol + $AlCl_3$ + HCl

Preparation of Hydrochloric acid reagent

About 5 mL of con. HCl is made up into 100 mL SMF using double distilled water to give 5% (v/v) solution.

The ethanolic stock solution is added to a drop of Aluminium chloride reagent and 5% HCl and was analysed UV-Vis spectra.

(5) Ethanol + NaOH

Preparation of Sodium hydroxide solution

About 1 gm of sodium hydroxide is dissolved in small amount of water and made up into 100 SMF (1 % Solution).

A drop of the Sodium hydroxide reagent is added to the ethanolic extract (5 mL) of the compound and was analysed UV-Vis spectra.

(6) Ethanol + NaOEt

Preparation of Sodium ethoxide reagent

Freshly cut metallic sodium (1.25 g) was dissolved cautiously added to 50 mL of ethanol and stored in a glass container.

A drop of the Sodium ethoxide reagent is added to the ethanolic extract (5 mL) of the compound and was analysed UV-Vis spectra.

(7) Boric acid

Anhydrous powdered reagent grade H_3BO_3 was used.

(8) Methanol

The compound (0.5 mg) is dissolved in 5 mL methanol and analysed for UV-Vis spectra.

The Methanolic solution of NaOAc, $AlCl_3$, $AlCl_3$ + HCl, NaOH, NaOEt were prepared by the above said procedure.

(9) Water

The compound (0.5 mg) is dissolved in 5 mL of DD water and analysed for UV-Vis spectra.

(10) Acetic acid

The compound (0.5 mg) is dissolved in 5 mL acetic acid and analysed for UV-Vis spectra.

Results and Discussion

An ethanolic solution of compound **I** produces band I maxima at 382 nm. The use of sodium acetate, a weaker base ionizes the most acidic C-7 hydroxyl group and the absorption shifts to 10 nm (392 nm). An addition of aluminium chloride forms complex between the C-4 carbonyl group and either 3-OH or 5-OH group and produces hypsochromic shift to 5 nm (377 nm). Upon addition of HCl into the $AlCl_3$ and HCl shows no change in absorption that suggests that the above formed complex is stable in acid. Sodium hydroxide solution also produces hypsochromic shift to 65 nm (317) due the ionization of all phenolic hydroxyl groups. However, sodium methoxide shifts the hypsochromic shift at 87 nm. Similar trends were observed when the solvent ethanol was changed into methanol. Additionally shift reagents of water and acetic acid observes the hypsochromic shift at 10 nm and 7 nm respectively. The versatile shift reagents used and their absorption are represented in **Table 1**. The UV- Vis spectra are presented in **Figure 2**.

Conclusion

UV – Vis shift reagents are powerful tool for the identification of flavonoids. In addition, the use of the above reagent has proven to be crucial in determining the substitution patterns. With this view we have studied the characteristic shift in absorption using versatile shift reagents for the 2-(3',4'-dihydroxyphenyl)-3,5,7-trihydroxy-8-((3'',4'',5''-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-2''-pyranil)oxy)-4H-4-chromenone, **I** which was discovered earlier in our laboratory. The shifts observed were interpreted and the localization of the position of the hydroxyl groups on compound **I** was detected by the use of the versatile shift reagents.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgement

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Tables and Figures

Figure 1: Compound **I**, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-8-((3,4,5-trihydroxy-6-(hydroxyl methyl)tetrahydro-2H-2-pyranil)oxy)-4H-4-chromenone

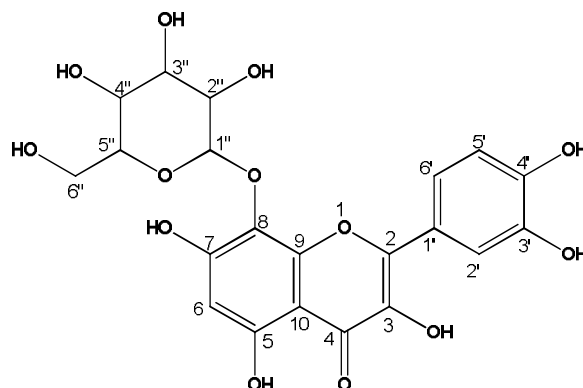
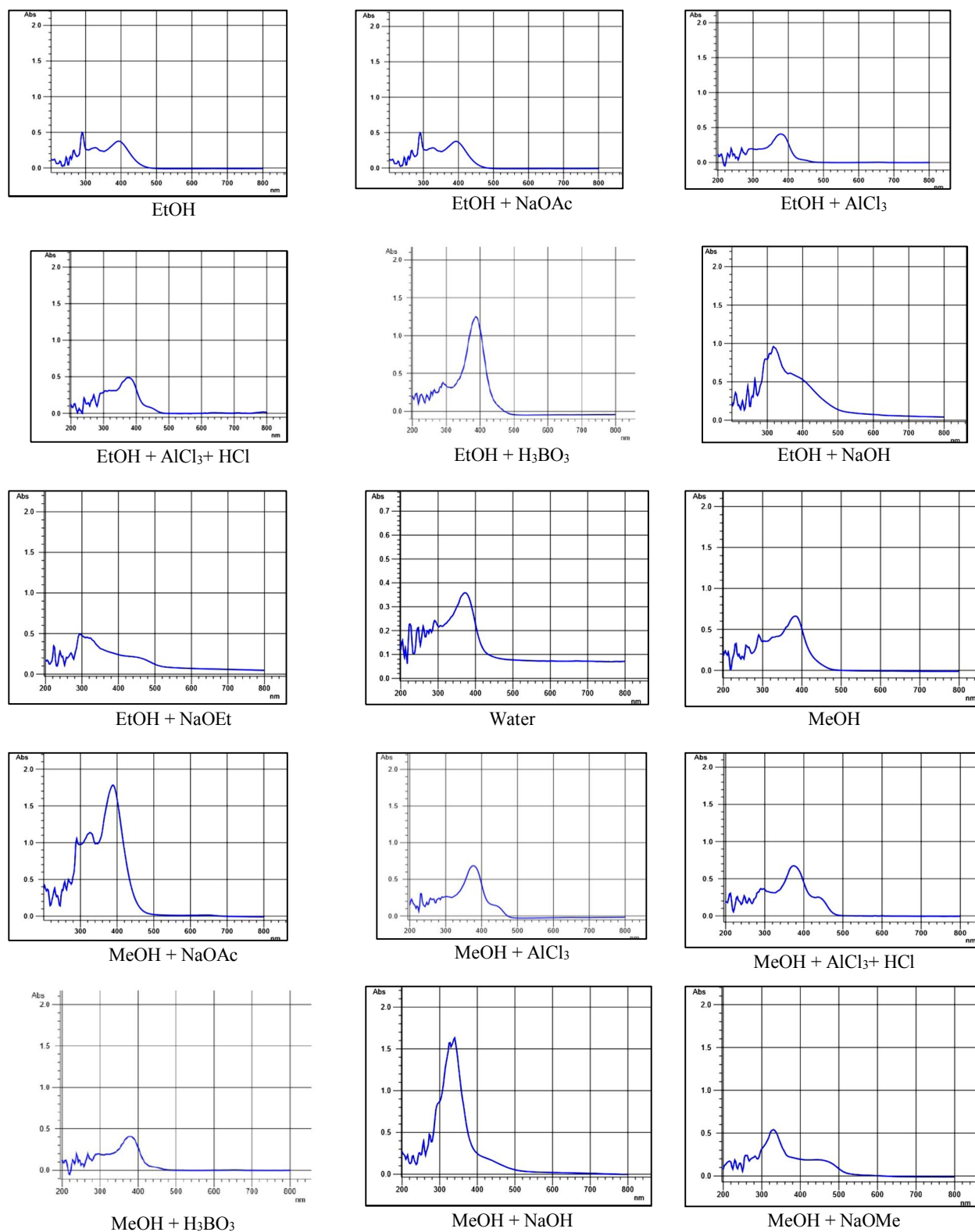


Figure 2: UV –Vis Spectra of compound **I** with different shift reagents

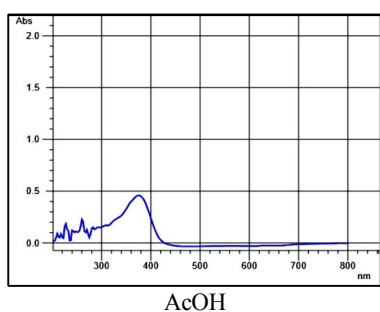


Table 1: Shift reagents used and their absorption

Shift reagent	$\lambda_{\max}(\text{nm})$	Shift reagent	$\lambda_{\max}(\text{nm})$
EtOH	382	MeOH	382
EtOH + NaOAc	392	MeOH + NaOAc	387
EtOH + AlCl ₃	395	MeOH + AlCl ₃	395
EtOH + AlCl ₃ + HCl	395	MeOH + AlCl ₃ + HCl	395
EtOH + H ₃ BO ₃	387	MeOH + H ₃ BO ₃	387
EtOH + NaOH	317	MeOH + NaOH	340
EtOH + NaOEt	295	MeOH + NaOMe	330
Water	372	Acetic acid	375