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NOTE

UV-Visible Spectral Investigation of Gardenin-A Using Shift Reagents

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A flavonoid, gardenin-A isolated from the gum resin of the plant *Gardenia gummifera* Linn, by thin layer chromatographic method is analyzed using UV-Visible spectral measurements. While chromatographic mobility and appearance give initial clues, the identification of the flavonoid is confirmed by the use of UV-Visible spectra, which is informative of their class and possible number of hydroxyl groups in the molecule. Addition of certain specific reagents to an alcoholic solution of Gardenin-A induced significant shifts in its UV spectrum. As a result, it has been possible to gain structural information from these spectra, which are considerably enhanced by the use of reagents that react with one or more functional groups on the flavonoid nucleus, Gardenin-A.

Key Words: Flavonoid, UV-visible Spectra, Shift reagents.

Gardenin-A (5-nydroxy-6,7,8,3′,4′,5-hexamethoxy flavone) belongs to the natural pigment flavonoids and has been isolated from the gum resin of the plant *Gardenia gummifera* Linn belonging to the family Rubiaceae. This plant is reported to be antiseptic, carminative, anthelmintic and useful in dyspepsia[1, 2]. Gardenin-A has been identified by chemical tests and its structure has been confirmed from ^{1}H NMR and ^{13}C NMR spectral data[3].

The part of the molecule in an organic compound that is responsible for the transitions giving rise to UV-Visible absorption is the chromophore. Placing a substituent on a chromophore may change the absorption by two different mechanisms—introduction of an entirely new transition and/or shifting the wavelength maxima of existing transition[4]. The UV-Vis spectrum of most flavonoids consists of two major absorption maxima, one of which occurs in the range 210–285 nm (band II) and the other in the range 300–400 nm (band I). In general terms, the band II absorption may be considered as having originated from A-ring benzoyl system and band I from B-ring cinnamoyl system. Highly oxygenated compounds absorb at longer wavelengths than those with fewer oxygenated substituents. Methylation or glycosylation causes hypsochromic shifts, particularly of band I. Acetylation of a flavonoid tends to nullify the effect of the phenolic hydroxyl groups on the spectra[5]. Thus, acetylation can be a valuable technique for locating alkoxy groups.

The UV-Vis spectra of flavonoid compounds are extremely informative of their class and possible number of hydroxyl groups in the molecule. However, additional measurements of spectra in the presence of a chelating ion or an anion capable of binding ortho-dihydroxy groups, give so much extra data that the structures of the

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compounds can be established beyond any doubt. The addition of sufficient base to allow all the free phenolic hydroxyl groups to ionize causes a shift of the bands to longer wavelengths. The extent of this bathochromic shift depends on the class of the compound and the overall substitution pattern.

A dilute solution of Gardenin-A in methanol was prepared, called plain solution. The UV-Vis Spectrum of Gardenin-A was recorded using UV-Vis SL 159 spectrophotometer. Two peaks were observed one at 323 nm (band I) and the other at 283 nm (band II) which is in agreement with the literature values. A strong solution of sodium acetate in alcohol was prepared and two drops of this solution were added to the plain Gardenin-A solution and the UV-Vis spectrum was again recorded. The peaks did not show any appreciable shift, with band I occurring at 326 nm and band II at 286 nm. Absence of shift in band II confirmed the absence of OH in the 7 position of the flavonoid nucleus Gardenin-A. To confirm the absence of ortho-dihydroxy groups, boric acid solution was added to Gardenin-A and sodium acetate solution and the UV-Vis spectrum was recorded. The spectrum revealed no appreciable shift, with the peaks remaining at 284 and 333 nm. On adding anhydrous aluminium chloride to the plain solution of Gardenin-A and recording the UV-Vis spectrum, shifts of 39 and 8 nm were observed in band I and band II respectively. This confirms the presence of chelated OH at 5 position. On addition of a few drops of conc. HCl to Gardenin-A and $AlCl_3$ solution, the UV-Vis spectrum remained unaltered with the bands appearing at 287 and 356 nm, confirming the absence of OH at 3 position. The wavelength maximum and abosrbance values corresponding to bands I and II are presented in Table-1.

TABLE-1
UV-VISIBLE SPECTRAL INVESTIGATION ON THE STRUCTURE OF GARDENIN-A

Reagents	Band II		Band I		Remarks
	λ_{max} (nm)	Absorbance	λ_{max} (nm)	Absorbance	
Gardenin-A	283	1.361	323	0.678	—
Gardenin-A + sodium acetate	286	2.078	326	0.846	No appreciable shift in band II confirms the absence of OH in the 7 position
Gardenin-A + sodium acetate + boric acid	284	1.923	333	0.658	No appreciable shift in band II confirms the absence of *o*-dihydroxy group
Gardenin-A + aluminium chloride	291	2.251	362	2.058	Appreciable shift of 39 nm in band I and 8 nm in band II confirms the presence of chelated OH at 5 position
Gardenin-A + aluminium chloride + conc. HCl	287	2.048	356	1.496	The shift is unaltered which confirms the absence of OH at 3 position

Sodium acetate is sufficiently basic to ionize hydroxyls located at 7, 3′ and 4′ positions and hydroxyls located elsewhere are unaffected. Thus band II is affected only if hydroxyl group is present at 7 position. This technique is, therefore, useful in deciding whether or not the 7-hydroxyl group in these classes of compounds is free or not. Addition of sodium acetate causes a bathochromic shift of about 8–20 nm of band II in compounds having free OH at 7. No appreciable shift in the band II position was observed which confirms the absence of OH in the 7 position in Gardenin-A.

For detecting the ortho dihydroxy groups, the reagents suitable are a mixture of sodium acetate and boric acid. If the *o*-dihydroxy system is in B-ring, a bathochromic shift of about 15–30 nm of band I is observed. A ring *o*-dihydroxy system gives rise to lesser shifts. In the present case, no appreciable shift was observed in band II on adding sodium acetate and boric acid, which confirms the absence of *o*-dihydroxyl groups in Gardenin-A.

Addition of anhydrous aluminium chloride to a methanolic solution of flavonoid gives an immediate yellow colour and is reflected in a bathochromic shift (shift to the longer wavelength) of the longer wavelength band. This is mainly due to the formation of a chelate between the carbonyl group and the adjacent hydroxy group at C-3 or if this is substituted, at C-5. When an *o*-dihydroxy group is present in the flavonoid together with a 5 or 3-hydroxyl, a double spectrum is formed. The spectrum has double peaks (with one inflection of lower intensity) for each band. On adding aluminium chloride to the plain Gardenin-A solution, an appreciable shift of 39 nm in band II was observed which confirms the presence of chelated OH at 5 position.

The anthocyanins containing ortho dihydroxy groups in the B-ring also give a shift of their visible peak with aluminium chloride. In this case, it is due to the formation of a chelate between the metal and the hydroxyl groups. Such chelates are also formed with flavonoids if the pH is not too acid (> 3). The interfering effects of these complexes are overcome by adding a little hydrochloric acid. For flavonoids having free OH at 3 position and for those having free OH at 3 and 5 positions a bathochromic shift of 50–60 nm of band II is observed with aluminium chloride/HCl. Flavonoids having an OH at 5 position and no OH at 3 position show a bathochromic shift of 35–55 nm of band I with aluminium chloride/HCl.

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