STUDY OF AN AL(III) COMPLEX WITH THE PLANT DYE BRAZILEIN FROM *CEASALPINIA SAPPAN* LINN AL(III) COMPLEX WITH BRAZILEIN FROM *CEASALPINIA SAPPAN* LINN

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Abstract

The structure of the complex formed between Al(III) (alum) and brazilein in aqueous solution was investigated using UV-visible spectroscopy. The molar ratio method and Job's method of continuous variation were applied to ascertain the stoichiometric composition of the complex in aqueous solution. A 1:2 complex was indicated by both methods. A structure for Al(brazilein)$_2$ was proposed and the calculated heat of formation of this complex, obtained by the semiempirical PM3 method, indicated that the proposed complex was a reasonable one energetically.

Keywords: brazilein, spectrophotometry, complexation, alum

Introduction

The red homoisoflavonoids, brazilin (6a*S-cis)* (7,11b-dihydrobenz[*b*]indeno[1,2-*d*]pyran-3, 6a,9,10,(6*H*)-tetrol) (Figure 1(a)) and brazilein (6a*S*-(6a,7-dihydro-3,6a,10-trihydroxy-benz[*b*] indeno[1,2-*d*]pyran-9(6*H*)-one) (Figure 1(b)), are components of the heartwood of the tree *Ceasalpinia sappan* Linn. (family Leguminosae) (Ferreira *et al.*, 2004). Homoisoflavonoids exhibit a flavonoid-like structure and can be classified as a sub-group of flavonoids. Flavonoids are a group of polyphenolic compounds that are widely distributed in plants and are used as pigments (DomŒnech-Carbó *et al.*, 2005). Brazilin is readily converted to brazilein by exposure to atmospheric oxygen and light. In Thailand, the aqueous extracts from the wood of *C. sappan* are generally used for the dyeing of silk, especially in the Northeast (Moeyes, 1993). The extracted dyes, which are mainly composed of brazilin and brazilein, give a beautiful red or pink colour to silk. However, this natural dye has poor fastness properties, and in order to try and overcome this metal ion-based mordants are used (Lemmens and Wulijarni-Soetjipto, 1992; Yan *et al.*, 2005). One such mordant generally used by villagers in Northeast Thailand is alum,

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which provides a source of Al(III) ions.

Alum ($KAl(SO_4)_2 \times 12H_2O$) is widely used as a mordant for dyeing keratin fibres with polyphenolic dyes to improve the fastness properties. The ability of metal ions to form very stable metal-dye complexes may be used to produce dyed protein fibres with superior fastness properties, especially towards washing and light (Christie, 2001).

Studies on the interaction between the crude dye extracted from *C. sappan* and various metallic ions, together with potential structures of the complex between the dye and Al(III) have been described (Kanazawa, 1991). However, the stoichiometry of the complex formed between Al(III) ions from alum with pure brazilein has not been determined. As part of our studies aimed at a better molecular understanding of mordant-natural dye interactions, we prepared pure brazilein by separate oxidation of extracted brazilin and then investigated the stoichiometric composition of the complex formed between brazilein and alum in aqueous solution with and without pH control. The molar ratio method was used for the spectrophotometric determination of the complex composition and Job's method was applied to confirm the results obtained with the molar ratio method. The results are reported in this paper, together with a proposed structure for the Al(III)-based complex.

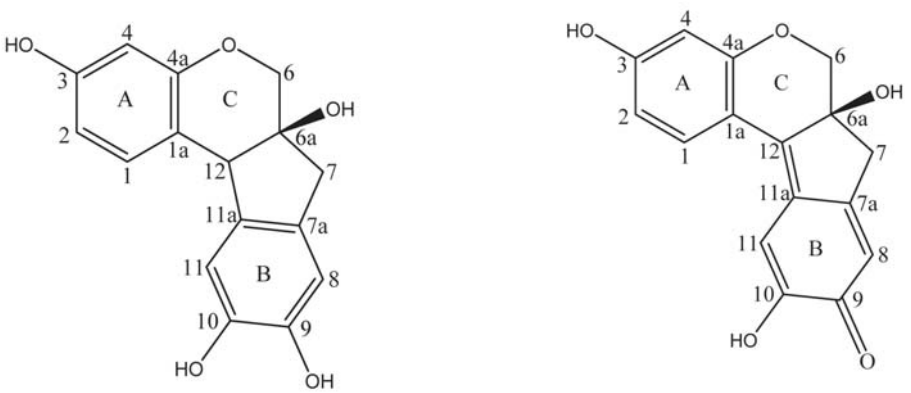
Materials and Method

Materials

Brazilein was prepared by oxidation of brazilin using iodine (Engels *et al.*, 1908) and brazilin was separated from the crude aqueous extract from the heartwood of *C. sappan*. The heartwood of *C. sappan* was obtained from Nakhon Ratchasima province, Thailand; a herbarium specimen No. KW001 (SUT) of the plant is lodged in the chemistry laboratory, Suranaree University of Technology, Nakhon Ratchasima. Alum, chloroform, ethanol, methanol, dimethyl sulfoxide (DMSO), acetone and iodine were obtained from Merck and UNICHROM. Solvents were A.R. grade.

Analytical Thin Layer Chromatography (TLC) and Column Chromatography

Analytical (TLC) was performed using aluminium backed sheets of Merck Silica Gel 60 F_{254} containing a fluorescent indicator, using a UV lamp (254 nm) to identify compounds on the plate. Column chromatography was performed using Merck Kiesel Gel 60 F_{254} (230 - 400 mesh) silica gel, with all solvent mixtures quoted as volume ratios.



(a) **(b)**

Figure 1. Chemical structure of (a) brazilin and (b) brazilein

^{1}H- and ^{13}C-Nuclear Magnetic Resonance (NMR) Spectra

All nuclear magnetic resonance (NMR) spectroscopy was performed on a Varian Unity 500 MHz spectrometer. Proton NMR (^{1}H-NMR) spectra and carbon NMR (^{13}C-NMR) spectra were acquired at 500 and 125 MHz respectively. Spectra for brazilin was recorded in acetone (CD_3COCD_3) with 0.05% tetramethylsilane (TMS) and for brazilein was recorded in dimethyl sulfoxide (CD_3SOCD_3), obtained from Cambridge Isotope Laboratories Inc., unless otherwise stated. TMS (0.00 ppm) was used as the internal standard.

Mass Spectrometry (MS)

Electron Impact (EI^+) low-resolution mass spectrometry was performed on a Shimadzu QP-5000 MAT-44 quadrupole spectrometer using the direct insertion technique. High resolution EI MS (for M^+) were run using a VG Autospec spectrometer operating at 70 eV and a source temperature of 250°C with PFK reference, and high resolution (ES) MS (for MH^+) with a Micromass Qtof 2 mass spectrometer using a cone voltage of 30 V and polyethylene glycol (PEG) as an internal reference. Electrospray mass spectrometry was performed on a Thermo Finnigan LTQ quadrupole ion trap (QIT) instrument equipped with an electrospray ionization (ESI) source.

Melting Points

Melting points were determined on a Reichert melting point apparatus. Temperatures are expressed in degrees Celsius (°C) and are uncorrected.

Optical Rotation

Optical rotations were determined on a Jasco digital polarimeter DIP-370.

Computational Modeling

Computer modeling was performed on a Silicon Graphics Fuel processor using PC Spartan Pro (Wavefunction, Irvine, Ca). Lowest energy conformers were determined by molecular modeling using MMFF94 force fields. The equilibrium geometries of the lowest energy conformers were then optimized using semi-empirical PM3 calculations.

Separation of Brazilin from Crude Extract of *C. sappan*

The dried heartwood (200 g) of *C. sappan* was chopped into small pieces, and extracted with boiling distilled water (2 L) for 1 h. The aqueous extract was removed by filtration and was first concentrated using a vacuum rotary evaporator and then freeze-dried in a vacuum freeze-dryer. The crude extract (500 mg) was then dissolved in methanol and pre-adsorbed on silica gel. Column chromatography on silica gel using $CHCl_3$-MeOH (15:1 to 7:1) as eluant gave brazilin as a crystalline reddish solid (100 mg; 20% based on the crude extract) (Kim *et al.*, 1997; Oliveira *et al.*, 2002). LRMS (EI) : *m/z* 286 [M^+], LRMS (ES) : *m/z* 285 [MH^+ for brazilein], HRMS (EI) : 286.0839, Calc for $C_{16}H_{14}O_5$, 286.0841, mp. : 127 - 131°C (Lit. 145 - 149°C, Kim *et al.*, 1997), $[\alpha]_D^{23}$: +69.8 (c 2.65×10^{-3}, DMSO).

Oxidation of Brazilin to Brazilein

Brazilin (100 mg) was dissolved in the minimum quantity of warm ethanol (*ca* 0.5 mL), mixed with hot distilled water (8 mL), and the clear solution then cooled to 60 - 70°C. A solution (1.6 mL) of iodine (33.8 g of iodine in 42.5 mL of ethanol) was added, and the mixture allowed to remain overnight at room temperature. The brazilein precipitated as a dark coloured powder. The precipitate was collected by filtration, washed with cold distilled water and then warm ethanol, and finally dried at room temperature to afford brazilein as a deep reddish-black solid (42 mg; 42%). LRMS (EI) : *m/z* 284 [M^+], LRMS (ES) : *m/z* 285 [MH^+], HRMS (EI) : 284.0678, Calc for $C_{16}H_{12}O_5$, 284.0685, mp. : 249 - 253°C (dec.) (Lit. 260 - 265°C (dec.), Kim *et al.*, 1997), $[\alpha]_D^{23}$: -1,125.5 (c 2.90×10^{-3}, DMSO).

The Composition of the Complex

The molar ratio method and Job's method were used to determine the composition of the Al(III) complex in solution by spectrophotometric means. For the molar ratio method, the brazilein stock solution (2.5×10^{-4} M) was prepared successively in methanol. Alum

solutions were prepared in distilled water. A concentration of 5.0×10^{-5} M of brazilein in water was diluted from the stock solution and kept constant while the alum concentration was varied from 0 to 2.0×10^{-4} M. In order to verify the results of the molar ratio method, the complex stoichiometry was also determined by Job's method. A series of solutions were prepared by keeping constant the total concentration of brazilein and alum, but their proportions were continuously varied by using the different volumes of brazilein (5.0×10^{-5} M) and alum (5.0×10^{-5} M) solutions. After initial mixing of the solutions, about 30 minutes was necessary to reach the complexation equilibrium. After this time, the absorption spectrum of each solution was recorded on a Cary UV-Vis-NIR model 500 scan spectrophotometer with quartz cells of 1 cm path length. For the experiments involving pH control of the solution, a buffer solution made up from an acetic acid-acetate buffer was used.

Results and Discussion

Properties of Brazilein

The EI mass spectrum of brazilein showed a $[M]^+$ at m/z 284 (HRMS (EI)): 284.0678, Calc for $C_{16}H_{12}O_5$, 284.0685. The UV-visible spectrum showed prominent maximum absorptions at 446 and 541 nm (DMSO). The melting point was 249 - 253°C (dec.) (Lit. 260 - 265°C (dec.), Kim *et al.*, 1997) and the specific optical rotation value was $[\alpha]_D^{23}$ -1,126 (c 2.90×10^{-3}, DMSO). The ^{1}H- and ^{13}C-NMR spectroscopic data and HMBC correlations confirmed the structure and were in good agreement with the literature data (Kim *et al.*, 1997).

UV-Visible Spectra of Dye in Aqueous Solution and at pH 4.5

The UV-Vis spectrum of brazilein in aqueous solution (Figure 2) showed three major absorption bands with wavelength maxima at 446 nm (band I), 541 nm (band II) and 276 nm (band III) respectively. Band I is considered to be associated with the absorption due to the B-ring in the cinnamoyl system, and band III with the absorption involving the A ring system. It was found that increasing the alum concentration decreased the absorbance of band I (446 nm) and also resulted in the appearance of a new band at 509 nm.

The effect of alum concentration on the visible spectra (λ_{max}) of brazilein without pH control is shown in Figure 2 and indicated a large bathochromic shift of 63 nm in band I of brazilein; the intensity of this new band at 509 nm increased with increasing concentration of alum. The bathochromic shift occurs on complexing to the

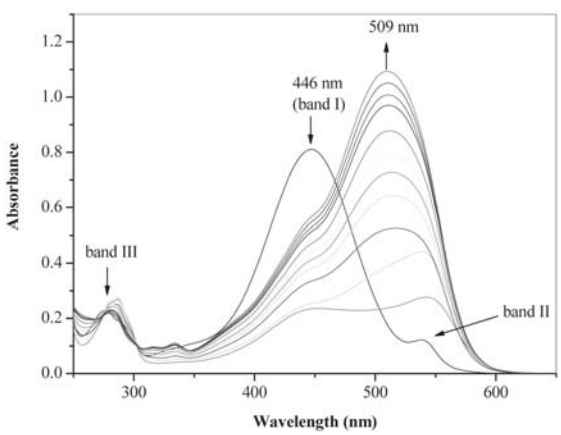


Figure 2. Electronic absorption spectra of brazilein (5.0×10^{-5} M) in aqueous solution in the absence and presence of alum (0 - 2.0×10^{-4} M)

aluminium ion through the lone pair of electrons present on the O donor atom. This electron pair donation stabilizes the excited state relative to the ground state leading to longer wavelength absorption maxima (Christie, 2001; Zollinger, 2003). Absorption spectra of brazilein in aqueous solution at pH 4.5 with different concentration of alum were similar to absorption spectra of brazilein and alum in aqueous solution without pH control and are not shown in this paper.

There are a number of particular technical advantages associated with the formation of coloured metal complexes. Generally, the metal complexes of organic ligands exhibit light-fastness properties better than those of the free ligands. This is due to the effect of coordination with a metal ion reducing the electron density at the chromophore, which in turn leads to improved resistance to photochemical oxidation. In addition, the larger size of the metal ion complex molecules compared with the free ligand generally gives rise to better washfastness properties through stronger interactions with the fibres (Christie, 2001).

Complex Stoichiometry of Brazilein and Alum in Aqueous Solution and at pH 4.5

In this study, the stoichiometry of the complex was determined by using the molar ratio and Job's methods. The molar ratio plots at 509 nm (λ_{max} of complex) is shown in Figure 3. An inflection in the molar ratio was observed at 0.50, indicating a stoichiometric ratio of 1:2 for the Al:brazilein complex. The Job plot at 509 nm (Figure 4) showed an inflection at 0.66 mole fraction of brazilein, indicative of a 1:2 Al:brazilein complex in agreement with the determination from the molar ratio method. From this stoichiometric ratio, a possible structure for the complex between brazilein and Al(III) ion is proposed, with an oxygen atom of the 9-carbonyl group and the anion produced by deprotonation of the 10-hydroxyl group, forming a chelate compound with the Al(III) ion as illustrated in Figure 5. Kanazawa (1991) also suggested a 1:2 Al:brazilein chelate (with water and nitrate ion as co-ligands) as one of three possible complexes from the crude aqueous extract of *C. sappan* heartwood with Al(III).

When the pH of Al(III)/brazilein solution was maintained at 4.5, it was found that the stoichiometric composition of the complex could not be determined from the molar ratio method. This might be due to the fact the constant H^+ concentration at the buffered pH of 4.5 suppresses the coordination properties of brazilein.

Al(III) ion has a coordination number of six and forms a complex with an octahedral configuration. In the case of brazilein, we

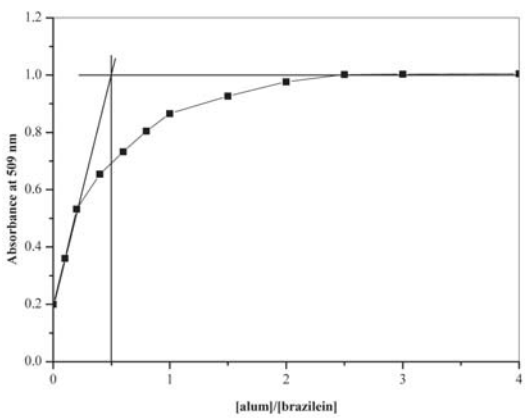


Figure 3. Absorbance versus [alum]/[brazilein] molar ratios plots at 509 nm without pH control

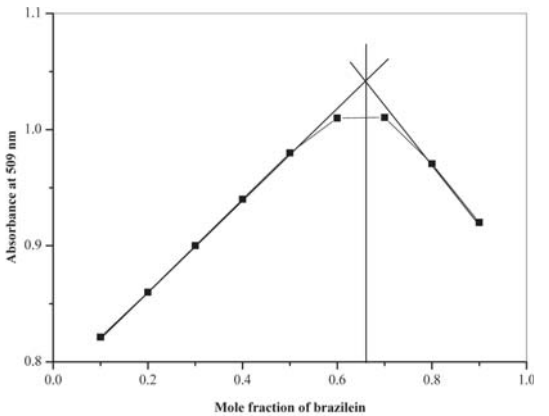


Figure 4. Absorbance versus the mole fraction of brazilein at 509 nm without pH control

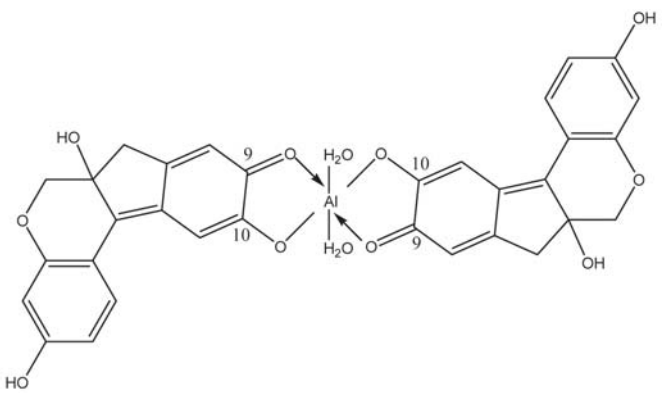


Figure 5. The proposed structure of the Al(brazilein)$_2$ complex

proposed that it coordinates as two bidentate ligands via the ionized 10-hydroxyl group and 9-carbonyl oxygen to Al(III), with two water molecules acting as co-ligands to complete the octahedral arrangement. The proposed structure of the Al-brazilein complex is shown in Figure 5. Computer-based molecular modeling was also carried out on this proposed structure of the complex by semiempirical PM3 calculations. Water molecules were added to maintain an octahedral environment for the complex ion. A heat of formation of -1,545.7 kJ mol^{-1} for the complex with H_2O molecules as co-ligands was found, consistent with a stable complex.

Conclusions

The interaction of brazilein and Al(III) (from alum) was studied by UV-Vis spectroscopy and the significant bathochromic shift observed for absorption band I in brazilein was consistent with complexation to the aluminium (III) ion. Using the molar ratio and Job's method, it was shown that the stoichiometric composition of the complex in aqueous solution was Al(brazilein)$_2$. The calculated negative heat of formation of the proposed complex indicated it was likely to be reasonably stable in aqueous solution.

Acknowledgements

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