Physio-chemical Studies on the Extract of *Rothmannia hispida*

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(Received 20th February, 1989; revised 22nd October, 1989)

Summary: A marine-blue-coloured dye has been extracted from the fruits of Rothmannia hispida. Based on its physical and chemical behaviour and spectral data the dye has been proposed to be a derivative of ortho-quinone, since it also formed a reddish orange quinoxaline derivative with o-phenylenediamine. This derivative got analysed correctly for its nitrogen content and indicated max at 720 nm in ethanolic solution. The dye forms complexes with a number of metal ions, particularly those of the transition series. This is indicated by significant shifts in the absorption maxima of pure dye and pure metal ions on complexation. The stability constants of dye complexes with Ti^{3+}, Cr^{3+}, Co^{2+} have also been estimated.

Introduction

Dyes of plant origin are quite numerous and have been reviewed recently [1]. Globally, however, interest in natural dyes appears to be on the decline, probably because of the relatively more accessible and may be cheaper synthetic dyes.

Nigeria like many other countries of the world has plants that abound in a number of natural dyes, most of which are known largely to the local population. One such plant, where fruits produce a dye stuff, is *Rothmannia hispida*. This plant grows as an erect shrub or tree and the crushed fruits give a marine-blue-coloured dye when suspended in water. The plant is found in Ghana, Cameroun, Zaira (Congo) and Southern Nigeria. Local people use the extract for fabric, leather ornament and textile dyeing as well as for artistic body dyeing for cultural festivities. A survey of the literature gives only scanty information and also reveals that virtually no scientific investigation have been carried out on the dye extract of *Rothmannia hispida* [2-4]. The work reported here, therefore, centres on the physico-chemical properties of the *Rothmannia hispida* dye extract.

Experimental

Absolute ethanol, ether, chloroform and carbon tetrachloride used were of the BDH Anala-R grade.

Rothmannia hispida fruits were supplied by a native farmer in Essien Itiaba, Akwa Ibom State (Nigeria).

Extraction of the Dye

The crushed seeds of the fruit (80 gm) of *Rothmannia hispida* were suspended in absolute ethanol (250 ml) in a flask (500 ml). The flask was stoppered and left standing in the laboratory fume chamber for one week at room temperature. A similar set-up was refluxed for 3 hours and then allowed to cool down to room temperature. In each case a marine-blue-coloured dye solution was obtained, which on removal of the solvent gave a dark-blue paste (0.5 gm). Purification was affected by redissolution in small amount of hot ethanol and reprecipitation on cooling. Filtration under vacuum and subsequent drying in desiccator for about one week yielded dark-blue crystalline product (0.32 gm), m.p. 106-109°C (leaving behind a very small amount of the unmelted substance, which also gave flame test for potassium ions).

The extraction with water also afforded the dye in a comparable yield, but recovery of the dry dye was difficult. Carbon tetrachloride and chloroform gave relatively lesser amounts (100 mg and 162 mg respectively) while ethereal extraction provided very poor yield (8 mg), when crushed seeds of this fruit (200 gm) were soxhlet extracted in separate experiments. The product from all these extracts had m.p. 106-109°C, which improved to 108-109°C (sharp) after two crystallization from chloroform. Consequently several soxhlet extracting experiments were run using chloroform only to collect sufficient amount of the natural dye. It was loaded on a silica gel column, but elution with hexane or ether did not practically afford any

material. However, chloroform eluted a bright blue dye, which after three crystallizations from carbon tetrachloride and chloroform (1:1) indicated sharp melting point at 108-109°C. Using Kieselgel-GF_{254} as adsorbent, both mono and bidirectional TLC of the product thus isolated gave only one spot, when CCl_4, $CHCl_3$ and CH_3OH were used as solvents. It may also be pointed out that the purified product from chloroform extract was used to carry out different tests, spectral analyses, and preparation of derivatives, except, where otherwise stated.

pH Measurement

A solution of the dye (0.1 gm) made in distilled water (100 ml) and pH measured using pye Unicam pH meter, model 292 MK2 gave a value of 6.5.

Test for Unsaturation

The dye (25 mg) was dissolved in water (5 ml) and 2% aqueous solution of potassium permanganate was added to it with vigorous shaking. The discharge of the potassium permanganate colour indicated the presence of an unsaturation in the dye, which was further supported by discharge of the bromine colour in carbon tetrachloride.

Test for Carbonyl Group

An aqueous solution of the dye (3 drops) were added to the Tollen's reagent in a clear test tube and heated on a water-bath for 2 minutes. No silver mirror characteristic of an aldehydic carbonyl group was obtained.

Test for Quinones [5]

The dye solution (2 ml) was added to an acidified potassium iodide solution (5 ml). The blue colour of the dye slowly changed to brown colour due to the liberation of iodine. This indicated the presence of a quinone.

Adding the alcoholic solution of the dye to an equivalent amount of o-phenylenediamine in alcohol, the reaction mixture warmed on the water bath for about 15 minutes, cooled and diluted with water, afforded a reddish orange quinoxaline derivative (m.p. 167-169°C) of the dye, which afforded 9.30% nitrogen content and indicated λ_{max} at 720 nm in ethanol. Its i.r. (KBr disc) spectrum indicated absorptions at 3350, 3120, 2912, 2890, 2850, 1660, 1635, 1610, 1580, 1510, 1425, 1410, 1360, 1230, 1060, 975, 820 and 770 cm^{-1}

Combustion Analysis and Molecular Weight Determination

The molecular weight of the dye ethanolic extract, was determined by the freezing point depression method, using water as the solvent. Its combustion analysis was also carried out. The results are shown in Table-1. However, the molecular ion of the product obtained from chloroform extract and using an EIMS 9 mass spectrometer was 224.

Spectroscopic Analysis

All infrared spectra were obtained on the Perkin Elmer model 577 grating infrared spectrometer as KBr discs of metal ion salt, dye, its derivatives and dye-metal ion complex. Similarly, the UV-visible absorption spectral data were obtained on the Cecil model 373 spectrophotometer. The n.m.r. spectrum of the dye in CCl_4 was obtained on the Varian T-60A NMR spectrophotometer.

Reaction of dye with Acids and Bases

The acid-base property of the dye was investigated with aqueous solutions (0.1 M) of HCl, H_2SO_4, NaOH and NH_3. A drop of the dye solution was added to each of the above reagents on a filter paper and no clear colour change was observed.

Determination of Absorption Maxima of dye, metal ions and dye-metal ion complexes [6]

These determinations were made with the Bausch and Lamb spectronic 20 UV-visible spectrometer, using 5% (W/W) ethanolic and aqueous solutions of each of dye and metal ion respectively. In addition, Job's method was followed to determine the dye-metal ion binding ratio in the case of Hg^{2+} and dye (assuming a molecular

Table 1: Results of Combustion Analysis and Molecular Weight Determination of the dye extract

Molecular weight found 224
Elemental composition (%)

	1*	2*
Carbon	41.71	41.52
Hydrogen	6.76	6.65
Oxygen	45.07	44.99
Nitrogen	1.19	1.26
Sulphur	0.09	0.00
Chlorine	0.10	0.23

*Results of two runs.

weight of 224) were used, with measurement of absorbance at 600 nm. The results are shown in Table-2.

Stability of the dye

20 ml of the aqueous solution of the dye was tested towards heat between 30-90° for 12 hours in an oven. Another 20 ml portion of the dye solution was exposed to sunlight for eight hours. No apparent change was noticed in both the cases.

Stability constant of dye-metal ion complex [6]

This was determined by assumption of a donor-acceptor pair for the dye-metal ion complex and measurement of the concentration of the complex executed spectrophotometrically. The results of this determination are shown in Table-3.

Results and Discussion

The molecular weight of the dye (extracted in ethanol), determined by freezing point, was found to be 234. Its combustion analysis revealed mainly three elements, C,H and O (Table-1). Moreover, the ethanolic extract gave positive tests for potassium (flame test), nitrogen (the Lassaigne's test) and nitrate anion (brown-ring test). Thus, the impurity in this extract may be attributed mainly due

Table 2: UV-visible absorption maxima (nm) of dye, metal ion and dye-metal ion complexes

System	λmax pure	λmax (complex)	Shift ($\pm$ nm)
Dye	330,480,600	-	-
Ti^{+3}	560	580	20
Cr^{+3}	620	610	10
Co^{+2}	490	520	30
Ni^{+2}	420	460	40

Table 3: Stability constants (K) and extinction coefficients (ε) of dye-metal ion complexes

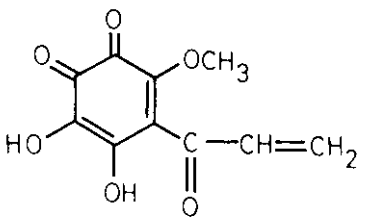
System	stability constants (K)	Extinction coefficients (ε)
Ti^{+3} : dye	99.8	1673
Cr^{+3} : dye	430	1300
Co^{+3} : dye	99.9	1820
Ni^{+2} : dye	71.4	4000

to potassium nitrate. The small amount of chlorine reported in Table-1 may be due to traces of potassium chloride. Such impurities are usually associated with natural products, which might have reached the finite through roots [7].

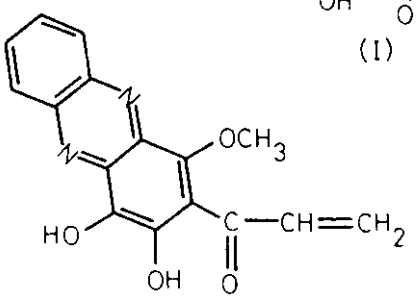
However, the dye extract using chloroform as a solvent did not give tests for K,N and Cl and gave only one spot on both mono and bidirectional TLC analysis. When analysed mass spectrometrically, gave molecular ion absorption corresponding to m/z 224. These observations support our contention that trace elements reported for the dye (ethanolic extract) in Table-1 are due to natural impurities.

The i.r. spectrum (KBr disc) of the dye (chloroform extract) showed significant absorptions at 3300 (OH-stretching), 3100 (C-H stretching, olefinic), 2890 and 2880 (C-H stretching saturated), 1680, 1650 and 1570 (C=O and olefinic), 1420

(scissoring motion of -CH_2), 1375 (symmetrical bending mode of CH_3 group), 1050 (C-O stretching) and 980 cm^{-1} (out of plane bending mode of the olefinic C- H). Thus, the proposed structure of the dye should have OH, C=O, C=C, CH_2 & CH_3 groups in its molecule [8]. Actually the dye discharged colour both of the potassiumpermanganate and bromine (in CCl_4) solutions had also suggested the olefinic nature of the dye. The hydroxy, carbonyl and olefinic functions have been manifested in its i.r. spectrum as well. Since the dye also gave a test for quinone (as given in the experimental section), the dye may be proposed to be a derivative of hydroxy-quinone. But *ortho* and *para*-quinones could be distinguished from their U.V. and visible spectra [5]. The *ortho*-quinone is known to show three bands in U.V. and visible region and the two longer wave-lengths are considered particularly useful in the characterisation of *ortho*-quinone. The dye showed three absorptions at 330 (log ε 2.5), 480 (log ε 1.83), and 600 nm (log ε 2.1). Thus, these values may be considered to correspond to a substituted *ortho*-quinone. The ^1H n.m.r. (CCl_4 60 MHz) signalled absorptions at δ 3.40 (3H,s), 3.65 (2H,m), 3.85 (1H,broad,s) which may account for OCH_3, CH_2 and CH protons in the molecule respectively. The two off-set deflections (in n.m.r.) at 176 and 224 Hz (which disappear by D_2O shake) are possibly for two non-equivalent hydroxyl groups. In conformity with physical and chemical behaviour, molecular weight determination (mass spectrometrically) and spectral data, the dye is, thus, tentatively assigned structure (I) having



(I)



(II)

molecular formula $C_{10}H_8O_6$ which agrees with its molecular ion absorption corresponding to 224. The proposal that this natural dye is a derivative of *ortho*-quinone is substantiated further from its formation of a reddish orange quinoxaline product (II) with o-phenylenediamine, which indicated λ_{max} at 720 nm due to extension of conjugation and gave expected i.r. absorptions. Moreover, determination of nitrogen for this quinoxaline derivative (II) corresponding to $C_{16}H_{12}N_2O_4$ was 9.30% as against 9.46% theoretically required for it. It is pertinent to point out that the proposed structure of the dye is also in line with many dyes of the natural origin [1]. However, the X-ray crystallographic analysis, which will be communicated later, is likely to provide unambiguous structure of this natural dye.

Complexation

As is evident from the experimental section, the almost neutral dye having a pH of 6.5 appears to be stable to heat (30-90°) and sunlight. It also appears that no acid-base indicator property is shown by the dye. However, it formed complexes with aqueous solutions of a number of transition metal ions, as evident from the relative shifts of their λ_{max} (Table-2). Some of them show red, while others show blue shifts. On the basis of the present investigations it is premature to speculate on the exact nature of the complexes. However, Job's method suggests the formation of a 1:1 complex in the case of Hg^{2+}. Moreover, the results of the stability constant determinations indicate a fairly strong complexation between the dye and Cr^{3+} (Table-3).

In view of the importance of complexation in dyeing process in the textile industry and in the analytical chemistry in general, the future investigations are likely to fill in the gaps.

Acknowledgement

Assistance from the Microanalytical laboratories of the Chemistry Department, University of Albarta and the Jagiellorian University, Institute of Chemistry Cracov, Poland, for the elemental analyses and the supply of *Rothmannia hispida* samples by Mr. O.O. Onyong are gratefully acknowledged.

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