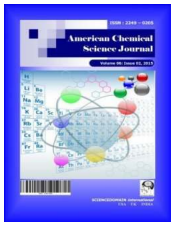


American Chemical Science Journal
11(1): 1-7, 2016, Article no.ACSJ.22393
ISSN: 2249-0205

SCIENCEDOMAIN *international*
www.sciencedomain.org

Synthesis of 4-Phenylazo Phenol from Anthocyanins of *Delonix regia* and *Hibiscus sabdariffa* Flowers

Oaikhena Z. Esezobor[1*], Bassey A. Maikasuwa[1], Terrumun A. Tor-Anyiin[1] and John O. Igoli[1]

[1]Department of Chemistry, University of Agriculture, Makurdi, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors TATA and JOI designed the research. Authors OZE and BAM performed the experiments and wrote the first draft of the manuscript. Author JOI did the NMR analysis. Authors JOI and OZE managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ACSJ/2016/22393
Editor(s):
(1) Nagatoshi Nishiwaki, Kochi University of Technology, Japan.
Reviewers:
(1) E. Yildizm, Cukurova University, Turkey.
(2) Mirela Kopjar, Josip Juraj Strossmayer University in Osijek, Croatia.
Complete Peer review History: http://sciencedomain.org/review-history/12261

Short Research Article

Received 30th September 2015
Accepted 28th October 2015
Published 10th November 2015

ABSTRACT

Anthocyanins extracted from flowers of *Delonix regia* and *Hibiscus sabdariffa* were coupled with diazotised aniline. Spectroscopic analysis revealed the resulting product to be 4-phenylazo phenol. This study provides a viable use for anthocyanins as an alternative source of phenol used in the synthesis of this dye.

Keywords: 4-phenylazo phenol; aniline; anthocyanins; Delonix regia; Hibiscus sabdariffa.

ABBREVIATIONS

^{13}C : *Carbon-13*
^{1}H : *Proton (hydrogen)*
d : *Doublet*
j : *Coupling constant*
m : *Multiplet*
s : *Singlet*
t : *Triplet*
td : *Triplet of doublet*
δ : *Chemical shift*

**Corresponding author: E-mail: esezoboroaikhena24@gmail.com;*

1. INTRODUCTION

Azo dyes are an important and versatile class of organic compounds with a variety of applications. The use of these dyes results from the combination of the properties of the azo group and several types of aromatic-substituted substrates that confer thermal and photochemical stability and an intense colour over the visible range [1]. Azo dyes are used in optical recording media [2], toners [3], ink–jet printing and oil–soluble light fast dyes [4]. They have been reported to show antineoplastic, antidiabetic, antiseptics, antibacterial, antitumour [5] and antitrypanosomal [6] activities. They are also involved in a number of biological reactions such as inhibition of DNA, RNA, protein synthesis, carcinogenesis and nitrogen fixation [5]. Azobenzene-based polymers can act as photosensitive, non-linear optical or photorefractive materials with ever-increasing field of application such as programmable optical interconnects, electro-optic modulation, coherent image amplification and holographic storage [7]. 4-Phenylazo phenol finds use as an intermediate in the synthesis of various azobenzene-based dyes. Condensation of 4-phenylazo phenol with haloacetic acid yields various halo-4-phenylazo phenoxyacetic acids which have been reported to show antimicrobial activities against bacteria such as *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli* and fungi such as *Candida albicans* [8]. Anthocyanins are a very interesting group of compounds due to their great diversity [9]. They comprise the largest group of water-soluble pigments in the plant kingdom [10] and are responsible for the orange, pink, red, magenta, purple, blue and blue-black floral colours [11]. *Delonix regia* has large brilliant red–orange coloured flowers that appear from April to August in Northern Nigeria [12]. The red–orange coloured flowers have been reported to be due to co–pigmentation between anthocyanins and other flavonoids [13]. *Hibiscus sabdariffa* is one of the species in the genus *Hibiscus.* In Nigeria, the red calyces are used to prepare a non–alcoholic drink called *zobo* [14]. Several phytochemicals including anthocyanins have been reported in the flowers of the plant [15–17]. Although the flowers of *H. Sabdariffa* are locally utilised, the economic potentials can still be enhanced while the flowers of *D. regia* constitute enormous biowaste during its flowering season.

Synthesis of azo dyes involves diazotisation of a primary aromatic amine and coupling with one or more nucleophiles such as aromatic amino or hydroxyl compounds [18]. The phenols and amines utilised here are usually obtained from the petrochemical industry. In this study, anthocyanins obtained from flowers have been employed as a source of phenol for the diazo coupling to produce a dye. The extraction of the anthocyanins and coupling with diazotised aniline to produce the dye as well as spectroscopic characterisation of the dye is reported.

2. MATERIALS AND METHODS

2.1 General

All reagents were from commercial sources. Solvents were redistilled before use. NMR (1D and 2D) experiments were carried out on a JEOL-LA-400 MHz FT-NMR. Fourier transform infrared (FT-IR) was recorded on a Shimadzu FTIR-8400S spectrophotometer and UV-Vis spectra was run on a Shimadzu UV2550 UV-VIS spectrophotometer. Mestrenova 9.0 was used to process the NMR data.

2.2 Extraction of Anthocyanins from the Flowers

Flowers of *Delonix regia* and *Hibiscus sabdariffa* were sun-dried and ground to powder. The ground flowers were macerated separately in 1% solution of HCl in methanol for 3 days and thereafter, the extracts were filtered and concentrated to a quarter of their original volumes using a rotary evaporator. The concentrated extracts were then transferred into a beaker and conc. HCl was added in ratio 1:5 v/v of extracts. The mixture was then placed in a refrigerator for precipitates to settle. The precipitates obtained were filtered without further purification and used in the next step of the synthesis.

2.3 Synthesis of the Dye

1.23 ml of aniline was dissolved in a mixture of conc. HCl (8.0 ml) and distilled water (8.0 ml) in a conical flask. The solution was immersed in an ice bath and cooled to 3℃. About 1.0 g of sodium nitrite was dissolved in 5 ml of distilled water in a separate flask and cooled to 3℃. The nitrite solution was slowly added to the aniline solution with constant stirring (ensuring that the temperature did not rise above 5℃) to produce the diazonium ion solution.

About 0.3 g of the plant extract (anthocyanin) was dissolved in 12 ml of 10% NaOH in a beaker

and cooled in an ice bath to 3℃. Then, the diazonium ion solution was slowly added while stirring and allowed to stand for 30 minutes also with stirring at intervals. The mixture was filtered and the dye product obtained was allowed to dry. The dye was purified by column chromatography on silica gel using ethyl acetate/methanol (2:1 v/v) as eluent.

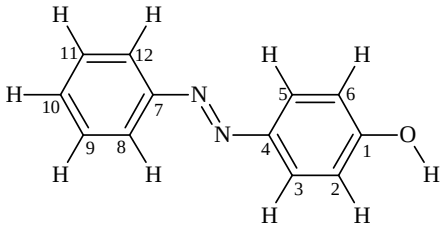
3. RESULTS AND DISCUSSION

3.1 Structure Elucidation

The dye (yield: 0.23 g; 76.7%) was obtained as black solid and gave one spot on TLC (R_f = 0.81) using ethyl acetate as the mobile phase. It gave UV absorption at λ_{max} 345 nm. Its FT-IR spectrum gave peaks at 3440 cm^{-1}, 1442 cm^{-1}, 1130 cm^{-1} corresponding to O–H stretch, N=N and C–N respectively. Its proton NMR spectrum (Appendix) gave five signals at δ_H 6.91 (2H, m), 7.38 (1H, m), 7.44 (2H, dd, *J* = 8.4, 6.4) and an overlap of two peaks at 7.81 (4H, m). The ^{13}C-NMR spectrum (Appendix) for the dye gave eight signals corresponding to three quaternary carbons at δ_C 160.8, 152.8 and 146.2 and five aromatic CH signals made up of four equivalent (2 x CH) at 115.5, 122.2, 124.7 and 128.8, and one CH at 130.0.

The ^{1}H-^{1}H correlation spectroscopy (COSY) spectrum for the dye showed that the protons at δ_H 6.91 were coupled to protons at 7.81 thus these protons are part of the same aromatic spin system. The protons at δ_H 7.44 were coupled to the proton at 7.38 and the protons at 7.81, thus these protons must also be part of another aromatic spin system. The ^{1}H-^{13}C heteronuclear single quantum coherence (HSQC) spectrum for the dye confirmed the attachment of the protons at δ_H 6.91, 7.38, 7.44 and 7.81 to the carbons at δ_C 115.5, 130.0, 128.8 and 124.7 respectively. Thus the rest of the carbon signals must be quaternary carbon atoms connected to the –N=N– linkage and the –OH group in the dye.

The long range (2*J*/3*J*) ^{1}H-^{13}C heteronuclear multiple-bond correlation (HMBC) spectrum for the dye confirmed the structure as follows: H-2 and H-6 were coupled (3*J*) to C-4, C-6 and C-2; H-3 and H-5 coupled (3*J*) to C-1 C-5 and C-3 while H-2 and H-6 were coupled (2*J*) to C-1; H-3 and H-5 coupled (2*J*) to C-2, C-4 and C-6. This further confirms their attachment to the same aromatic ring. Similarly the spectrum revealed H-8 and H-12 coupled (3*J*) to C-10, C-12 and C-8; H-9 and H-11 were coupled (3*J*) to C-7, C-11 and C-9 while H-10 was coupled (3*J*) to C-8 and C-12; H-8 and H-12 were coupled (2*J*) to C-7, C-9 and C-11; H-9 and H-11 coupled (2*J*) to C-8, C-10 and C-12. Finally H-10 was coupled (2*J*) to C-9 and C-11. This also indicates the attachment of these protons to the same aromatic ring. From the spectral data and comparison to literature reports [7, 19], the dye was identified as 4-phenylazo phenol (**1**).



(1)

Selected COSY and HMBC (3*J*) correlations for the dye are shown in Fig. 1.

The NMR spectral data for the dye compared to literature reports are given in Table 1.

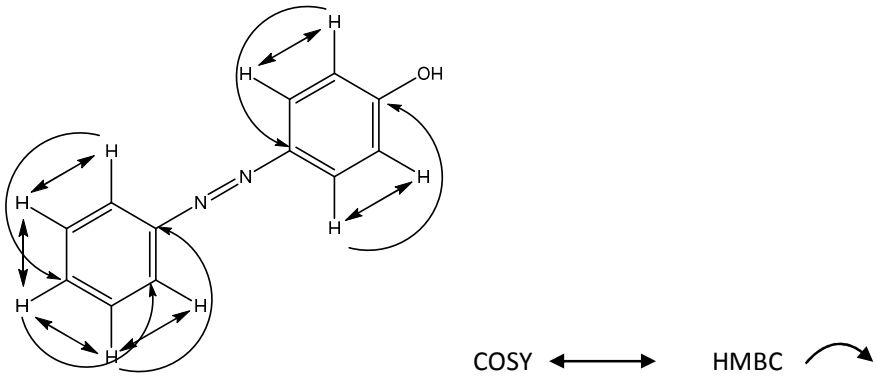


Fig. 1. Selected 2D correlations for the dye

Table 1. NMR data for the dye and literature reports

Pos.	^{1}H–NMR (δ)				^{13}C–NMR (δ)			
	Experimental value		Ohlsson et al. [19]	Parker et al. [7]	Experimental value		Ohlsson	Parker
	DRFD (DMSO)	HSFD (MeOD)			DRFD (DMSO)	HSFD (MeOD)	et al. [19]	et al. [7]
1			–	–	161.6 (C)	160.8 (C)	158.2	162.3
2	6.74 (2H, m)	6.91 (2H, m)	6.94 (2H)	6.92 (2H, d)	116.4 (CH)	115.5 (CH)	115.8	116.8
3	7.56 (4H, td, *J* = 7.05, 1.87)	7.81 (4H, m)	7.91 – 7.85 (4H, m)	7.76 – 7.88 (4H, m)	125.3(CH)	124.7 (CH)	125.0	126.1
4	–	–	–	–	145.6 (C)	146.2 (C)	147.1	147.6
5	7.56 (4H, td, *J* = 7.05, 1.87)	7.81 (4H, m)	7.91 – 7.85 (4H, m)	7.76 – 7.88 (4H, m)	125.3 (CH)	124.7 (CH)	125.0	126.1
6	6.74 (2H, m)	6.91 (2H, m)	6.94 (2H)	6.92 (2H, d)	116.4 (CH)	115.5 (CH)	115.8	116.8
7	–		–	–	152.6 (C)	152.8 (C)	152.6	154.2
8	7.56 (4H, td, *J* = 7.05, 1.87)	7.81 (4H, m)	7.91 – 7.85 (4H, m)	7.76 – 7.88 (4H, m)	122.5 (CH)	122.2 (CH)	122.5	123.5
9	7.31 (2H, t, *J* = 7.53)	7.44 (2H, dd, *J* = 8.35, 6.42)	7.53 – 7.48 (2H, m)	7.34 – 7.56 (4H, m)	129.8 (CH)	128.8 (CH)	129.0	130.2
10	7.25 (1H, t, *J* = 7.09)	7.38 (1H, m)	7.47 – 7.42 (1H, m)	–	130.9 (CH)	130.0 (CH)	130.4	131.4
11	7.31 (2H, t, *J* = 7.53)	7.44 (2H, dd, *J* = 8.35, 6.42)	7.53 – 7.48 (2H, m)	7.34 – 7.56 (4H, m)	129.8 (CH)	128.8 (CH)	129.0	130.2
12	7.56 (4H, td, *J* = 7.05, 1.87)	7.81 (4H, m)	7.91 – 7.85 (4H, m)	7.76 – 7.88 (4H, m)	122.5 (CH)	122.2 (CH)	122.5	123.5

DRFD – Delonix regia flower dye; HSFD – Hibiscus sabdariffa flower dye; DMSO – dimethyl sulphoxide; MeOD – methanol; $CDCl_3$ – chloroform

Control experiments using a similar extract of the yellow flowers of *Cascabela thevetia* and a 10% solution of NaOH without the anthocyanin extracts added did not yield any dye products and confirms that the source of the phenol moiety in the dye is the anthocyanins and diazotised aniline did not undergo a substitution reaction in the presence of the base to form the phenol.

4. CONCLUSION

The coupling of diazotised aniline with anthocyanins obtained from the flowers of *Delonix regia* and *Hibiscus sabdariffa* has led to the synthesis of 4-phenylazo phenol. This study provides a viable use for these flowers via the use of their anthocyanin constituents in the partial synthesis of a useful and common azo dye.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

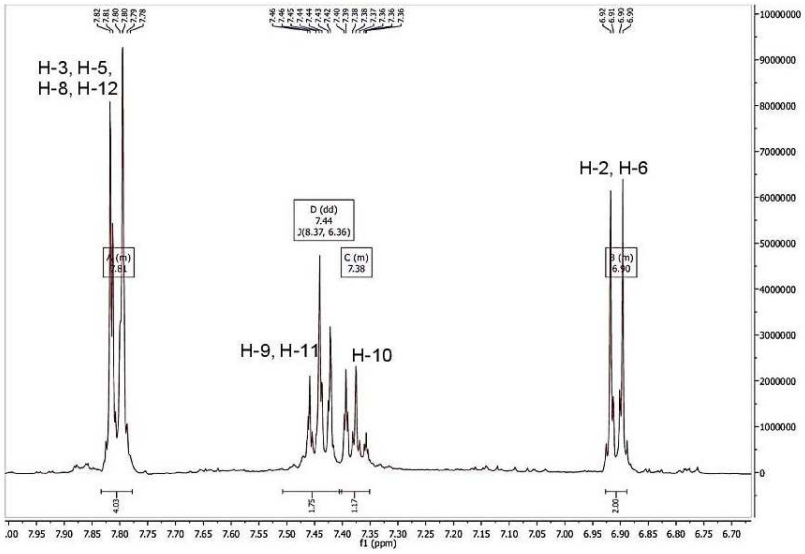
1. Hameed AS. Characterization and thermal decomposition of indolylidene aniline azo-dyes derivatives. Jordan J. Chemistry. 2007;2(2):133-144.
2. Samichet F, Ahmad MT, Hooshang H, Hojatollah K. A synthesis of some new 4–arylidene–5(4H)–oxoazoloneazo dyes and an evaluation of their solvatochromic behaviour. ARKIVOC. 2008;14:115-123.
3. Kirkan B, Gup R. Synthesis of new azo dyes and copper (II) complexes derived from barbituric acid and 4–Aminobenzoyl-hydrazone. Turk. J. Chem. 2008;32:9-17.
4. Otutu JO. Synthesis and application of azo dyes derived from 2–amino–1, 3,4–thiadiazole–2–thiol on polyester fibre. IJRRAS. 2013;15(2):292-296.
5. Ajani OO, Akinremi EO, Ajani OA, Edobor-Osoh A, Anake UW. Synthesis and spectroscopic study of naphtholic and phenolic azo dyes. Phy. Rev. and Res. Int. 2013;3(1):28-41.
6. Igoli JO, Blackburn G, Gray AI, Sutcliffe OB, Watson DG, Euerby MR, Skellern GG. Chromatographic and spectroscopic analysis of the components present in the phenanthridinium trypanocidal agent isometamidium. Anal. Bioanal. Chem. 2015;407(4):1171-80.
7. Parker RM, Gates JC, Rogers HL, Smith PGR, Grossel MC. Using the photo-induced reversible refractive-index change of an azobenzene co-polymer to reconfigure an optical Bragg grating. J. Mater. Chem. 2010;20:9118-9125.
8. Moanta A, Radu S. New phenoxyacetic acid analogues with antimicrobial activity. Rev. Chim. (Bucureºti) 2008;59(6): 708– 711.
9. Goulas V, Vicente RA, Manganaris AG. Structural diversity of anthocyanins in fruits. In: Anthocyanins: structure, biosynthesis and health benefits. New York: Nova Sciences Publishers, Inc; 2012.
10. Ponmozhi P, Geetha M, Kumar SM, Devi SP. Extraction of anthocyanin and analysing its antioxidant properties from *Pithecellobium dulce* fruit pericarp. Asian J. Pharm. Clin. Res. 2011;4(1):41-45.
11. Davies KM. Plant pigments and their manipulation. Oxford: Blackwell Publishing; 2004.
12. Dakku UH. *Delonix regia:* An important naturalized bee plant in northern Nigeria. Bee World. 2011;71–72.
13. Padma SV. Delonix: A source of natural dye; 2012.

    Available:http://www.nptel.ac.in/courses/116104046/33.pdf (Accessed 12 May 2015)
14. Ijeomah AU, Ugwuona FU, Abdullahi H. Phytochemical composition and antioxidant properties of *Hibiscus sabdariffa* and *Moringa oleifera*. Nigerian J. Agric Food Env. 2012;8(1):10-16.
15. Fasoyiro SB, Babalola SO, Owosibo T. Chemical composition and sensory quality of fruit-flavoured Roselle (*Hibiscus sabdariffa*) drinks. World Journal of Agricultural Sciences. 2006;1(2):161-164.
16. Mohamed BB, Sulaiman AA, Dahab AA. Roselle (*Hibiscus sabdariffa* L.) in sudan, cultivation and their uses. Bull. Env. Pharm. Life Sc. 2012;1(6):48-54.
17. Puro K, Sunjukta R, Samir S, Ghatak S, Shakuntala I, Sen A. Medicinal uses of Roselle plant (*Hibiscus sabdariffa* L.): A mini review. Indian J. Hill Farm. 2014; 27(1):81-90.

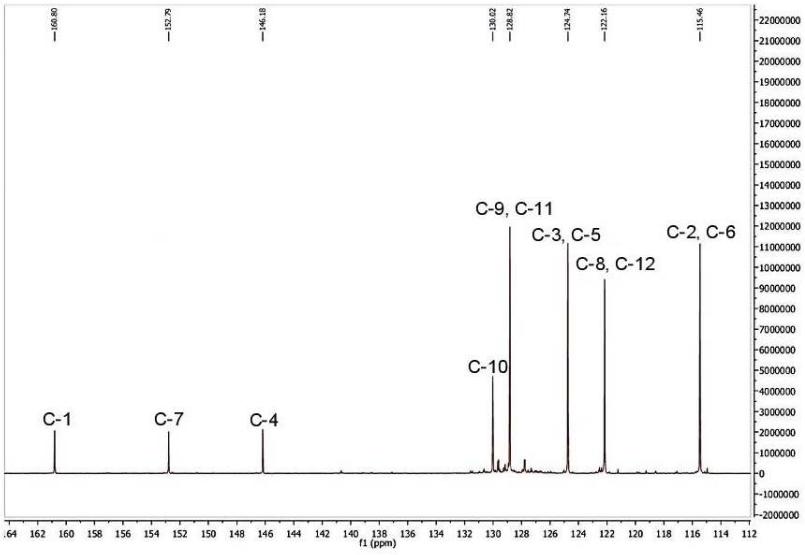
18. Zollinger H. Color chemistry: Synthesis, properties and applications of organic dyes and pigments. 3rd Revised Ed. Weinheim: Wiley-VCH; 2001.

19. Ohlsson J, Wolpher H, Hagfeldt A, Grennberg H. New dyes for solar cells based on nanostructured semiconducting metal oxides: Synthesis and characterisation of ruthenium (II) complexes with thiol-substituted ligands. J. Photochemistry and Photobiology A: Chem. 2002;148:41-48.

APPENDIX



An expanded ^{1}H-NMR (MeOD) spectrum for the dye (region δ_H 6.70 - 8.00 ppm)



An expanded ^{13}C-NMR (MeOD) spectrum for the dye (region δ_C 112 – 164 ppm)

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/12261