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Der Chemica Sinica, 2010, 1 (2): 21-28

Pelagia Research Library
ISSN: 0976-8505
CODEN (USA) CSHIA5

Isolation and characterization of flavonoids chalcones and anthocynidines from bridelia ferruginea benth

Donatus Ebere Okwu*, Nneka Ukanwa

Department of Chemistry
Michael Okpara University of Agriculture, Umudike
P.M.B. 7267, Umuahia, Abia State, Nigeria

ABSTRACT

Chalcones and anthocyanidines represents essential groups of natural products which possess wide range of pharmacological activity such as antibacterial, antitumor, anticancer, anti-tubercular, anti-inflammatory, antioxidant and anti-malarial properties. From the ethanolic extract of the leaves of Bridelia ferruginea Benth, two flavonoids bioactive compounds 6, 4^{l} dihydroxy 3^{l} propen chalcones and 4^{l} propenoxy 7–hydroxy anthocyanidines were isolated. The structures of these flavonoids were characterized on the basis of their IR, NMR and MS spectroscopic data.

Key words: *Bridelia ferruginea*, Anthocyanidines, Chalcones Biological activity.

INTRODUCTION

The flavonoids belong to one of the most bioactive compounds which naturally exist in the plant kingdom. Different naturally occurring flavonoids have been described and subcategorized into flavones, flavans, flavanones, isoflavonoids, chalcones, aurones and anthocyanidines [1,2]. These flavonoids have remarkable biological activities, including inhibitory effects on enzymes, modulatory effect on some cell types, protection against allergies, antiviral, anti-malarial, antioxidant, anti-inflammatory and anti-carcinogenic properties [1,2].

Bridelia ferruginea Benth belongs to the Euphorbiacea family. It is a tropical shrub which sometimes grows up to 18 m high and about 1.5 m in girth [3]. This plant is extensively used in herbal medicine in Nigeria.

In phytomedicine, the bark infusion is used as a purgative and vermifuge [4]. The latex from the bark and the leaves are used to prepare soup used to aid lactation by nursing mothers [5]. The leaf decoction has been used extensively as a wash for fever. The bark leaves and root extracts of *B. ferruginea* are ingredients of the Yoruba ‘agbo’ infusion and are used in the preparation of a

popular mouth wash and as a remedy for thrush in children [6]. Extracts from the roots and leaves is used to cure piles, diarrhea and dysentery [7].

In Southwestern Nigeria, an infusion of shredded leaves is highly valued for washing cuts, sores, wounds and burns. The juice of the crushed leaves and powdered dried leaves are used as poultices on wounds and inflammations while the stem bark extract is used to expel roundworms and is given for the treatment of cystitis [6]. It has been found [8] that an aqueous extract of the leaves has antibacterial action, and the alcoholic extract is very more effective. The decoction of the roots is used to treat gonorrhea and *Candida* infections [8]. The roots, barks and leaves have astringent properties and are used as lotion to treat toothache or as an infusion in diarrhea, piles and dysentery [8]. The stem barks, roots and leaves extracts are used as ingredients for the preparation of a remedy for leprosy, cardiac diseases, thrombosis, atherosclerosis, lumbago and general fatigue. Extracts from the leaves, roots and stem bark are used to treat elephantiasis of the scrotum [7]. The stem bark are pounded into powder and used to treat hemorrhoids.

In Northern Nigeria, the roots are employed as a remedy for oedma, rheumatism and arthritis [5]. In Southern Nigeria, the plant is considered sacred and is featured in certain rituals and ceremonies. *B. ferruginea* is used as an antidote to arrow poison [6].

In Nigeria, indigenous people traditionally use *Bridelia ferruginea* to maintain their health. *Bridelia ferruginea* plant has reservoirs of many secondary metabolites which exhibits some medicinal properties. All the organs of the plant are rich in tannins and flavonoids [6]. Flavonoids and biflavonoids such as apigenin and kaemferol were isolated together with their glucosides from methanolic extract of the plant [6]. Other bioactive compounds isolated from *B. ferruginea* include quercetin, rutin, myricetin, gallocatechin, epigallocatechin and their glucosides [8].

Herein we report the isolation and characterization of a chalcone 6, 4^{l} dihydroxy 3^{l} propen chalcones together with the isomeric anthocyanidines 4^{l} propenoxy 7–hydroxy anthocyanidines from the leaves of *Bridelia ferruginea.*

MATERIALS AND METHOD

General Experimental Procedure

The IR spectra were determined on a Thermo Nicolet Nexus 470 FT–IR spectrometer. UV spectra were recorded on Shimadzu 160A spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400 FT spectrometer for ^{1}H NMR and 75 FT spectrometer for ^{13}C NMR, using TMS as internal standard. Chemical shifts are expressed in parts per million (ppm).

LC-ESIMS spectra were determined in the positive ion mode on a PE Bio-system API 165 single quadruple instrument, HRESIMS (positive ion mode) spectra were recorded on a Thermo finniga MAT 95 XL mass spectrometer. Column chromatography was carried out with silica gel (200 – 300 mesh) and to monitor the preparative separations, analytical thin layer chromatography (TLC) was performed at room temperature on pre-coated 0.25mm thick silica gel 60 F_{254} aluminum plates 20 x 20cm Merck, Darmstadt Germany.

Plant Materials

The fresh leaves of *Bridelia ferruginea* were collected from an uncultivated farmland in Umudike, Abia State, Nigeria on 6th February, 2008. The plant samples (leaves, fruits and seeds)

were identified by Dr. A. Nmeregini of Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture Umudike, Nigeria. A voucher specimen No: BF/553 has been deposited at the Forestry Department, Herbarium of the University.

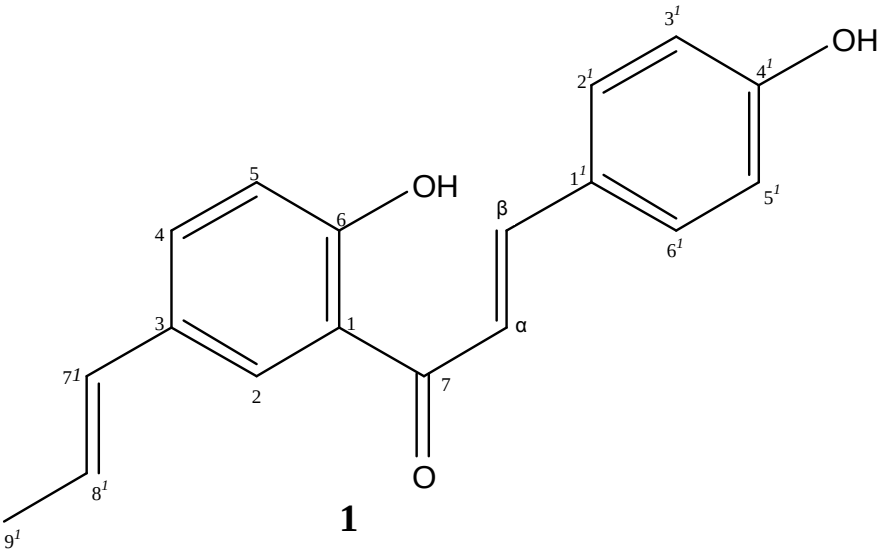
Extraction and Isolation of Plant Materials

Plant materials were treated and analyzed at the Chemistry Laboratory, Michael Okpara University of Agriculture Umudike, Nigeria. The leaves (1 kg) were dried on the laboratory bench for 10 days. The dry samples were milled and ground into powder (912 g) using Thomas Wiley Machine (Model 5 USA). The powdered plant sample (500g) was packed into a Soxhlet apparatus (2L) and extracted exhaustively with 1000 ml ethanol for 24 hours. The ethanol extract was concentrated using a rotary evaporator at room temperature and left on the laboratory bench for 3 days to obtain a dark green pigment (50.3g). The column was packed with silica gel and the green pigment placed on top and eluted with methanol, chloroform and petroleum ether (20: 30: 50) to afford a yellow orange powder (0.80g). The yellow orange powder was re-crystallized from hexane to afford compound **1**, yellow orange powder (0.52g), Rf 0.72, IR Vmax 3401 cm^{-1} (OH), 2926 cm^{-1} (CH_2), 1725 cm^{-1} (C=O), 1614 cm^{-1} (C=C) aromatic. HREIMS m/z 280.1631 calculated for $C_{18}H_{16}O_3$ (m/z 280) calculated for $C_9H_9O_2$ (m/z 149.) UV: λmax (MeOH) 370 nm ^{1}H NMR and ^{13}C NMR were presented in Table **1**.

Compound **2**, yellow crystalline powder (0.46g), Rf 0.63 IR Vmax 3401 cm^{-1} (OH), 1614 cm^{-1} (C=C aromatic) and 1070 (C–O). UV λmax (MeOH): 540 nm. HREIMS m/z 280.1629 calculated for $C_{18}H_{16}O_3$ (m/z 280) with base peak at 149.0208 calculated for $C_9H_9O_2$ (m/z 149.) ^{1}H NMR and ^{13}C NMR were presented in Table **1**.

RESULTS AND DISCUSSION

The ethanol extract of the leaves of *B. ferruginea* was fractionated by silica gel column chromatography to afford compounds **1** and **2**. Compound **2** can be considered either direct derivatives or biosynthetic analogues of compound **1** (Scheme 1). A prenyl unit and para-substituted benzene ring are two common occurring natural product structural elements present in this molecule forming the rings A and B of compound **1**. Compound **1** was identified as chalcone 6, 4^l dihydroxy 3^l propen chalcones. The IR spectrum showed the hydroxyl group at 3393 cm^{-1} and α, β – unsaturated carbonyl group at 1725 cm^{-1} and the aromatic groups at 1616 cm^{-1}.



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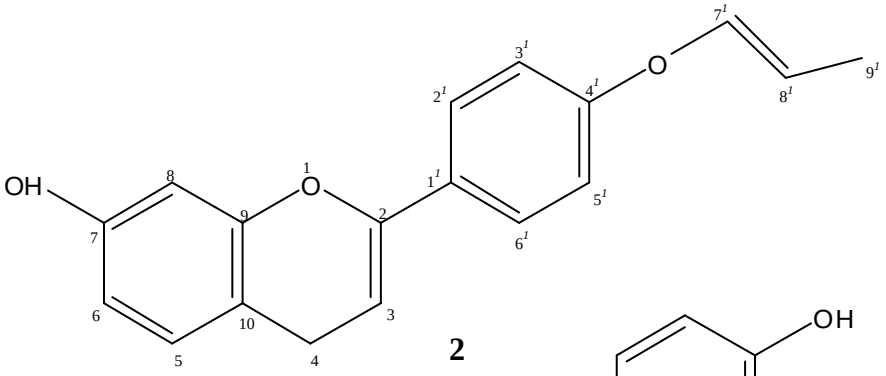

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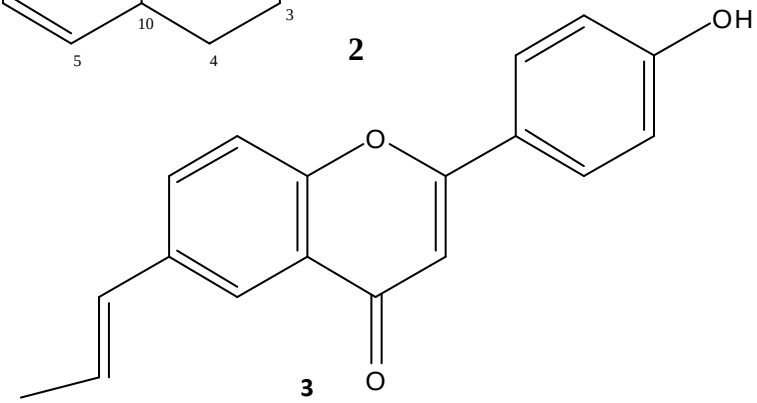

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The UV data was at λmax 370 nm supporting the presence of a chalcone [1]. The EIMS spectrum of compound **1** revealed [m+] ion peak at m/z 280.163, indicating the molecular formula $C_{18}H_{16}O_3$ (cal. m/z 280). Further peaks were obtained at m/z 279.1588 due to proton migration. The compound undergoes α – cleavage from the carbonyl to produce the base peak m/z 149.0324 calculated for $C_9H_9O_2$ m/z 149 (Figure **1**). The ^{1}H NMR spectrum of compound **1** showed the presence of methyl groups at δH 1.27960 (3Hd) and seven aromatic protons at δH 7.61050 (1Hs), 6.66944 (1Hd), 6.69722 (1Hd), 7.61050 (2Hd) and 7.61050 (2Hd). The presence of the isopropen group was indicated by a methyl group at δH 1.27960 (3Hd) and olefin protons at δH 4.52279 (1Hd) and 4.46730 (1Hm). The two hydroxyl protons attached to C_6 and $C_4{}^l$ appeared at δH 5.08174 and 5.32788. The ^{13}C NMR spectrum of compound **1** showed the carbonyl carbon at δC 169.320 while the α and β-carbons appeared at δC 125.392 and 145.980 respectively. Compound **1** was identified as 6, 4^l dihydroxy 3^l propen chalcones. This identification is based on the physical data UV, IR, ^{13}C and ^{1}H NMR spectroscopy which is in accord with literature data (Chedwick *et al* 2004, [1,9]. The prenylated flavonone 4^l hydroxy 6–propen flavonone **3** is an artifact which are biosynthetically formed by spontaneous isomerization ion of the parent natural product 4, 2^l dihydroxy 3^l propen chalcones **1** which contain two phenyl rings. Conjugate ring – closure of the chalcone resulted in the formation of the flavonoids, the three ring structure of a flavones. The metabolic pathway continuous through a series of enzymatic modifications to produce flavonones. By retro-Diels-Alder rearrangement that occurred as a result of cyclization compound **2** was obtained from the same plant as an isomer of compound **1** (Scheme **1**).

Compound **2** isolated as a yellow crystal solid was identified as 4^l propen dihydroxy–7–hydroxyl anthocynidines was assigned the molecular formula m/z 280.1629 calculated for $C_{18}H_{16}O_3$ (m/z 280) with base peak at m/z 149.0208 calculated for $C_9H_9O_2$ (m/z 149). Lost of a proton from the parent ion produces the peak at m/z 279.1582 calculated for $C_{18}H_{16}O_3$ (m/z 279), while detachment of the propenoxy group give the peak at m/z 57.0707 calculated for C_3H_5O (m/z 57). Fragmentation of the flavonoid heterocyclic nucleus with the hydroxyl group produces the base

peak m/z 149.0224 calculated for $C_9H_9O_2$ (m/z 149) (Figure **2**). The results of this molecular formula analyzed showed that the chalcone compound **1** and anthocyanidine compound **2** are isomers. The IR spectrum of compound **2** revealed hydroxyl, aromatic and ether bands at 3401 cm^{-1}, 1614 cm^{-1} and 1070 cm^{-1} respectively. It has UV absorption band at Vmax 520 nm. The absorption of UV band at 520 nm indicates that compound **2** is anthocyanidine flavonoid [1].
Analysis of the ^1H NMR spectrum of compound **2** is shown in Table **1**. Seven signal protons were observed at δH 1.96622 (3Hs), 1.66660 (1Hm), 1.68038 (1Hd), 5.08174 (OH brs) and 7.05839 (7 Hs). The $H_8{}^l$ olefin proton signal is reported to be multiplet and appeared at δH 4.2267 which correlates to the ^{13}C NMR of olefin peaks at δC 148.006 (CH) and δC 143.812 CH for $H_7{}^l$. The methyl proton signals of $C_7{}^l$ appeared as doublet at δH 1.28688 while the ^{13}C NMR of the methyl group appeared at δC 24.472. The hydroxyl protons at C_7 appeared at δH 4.84209 (brs). Combining the information obtained from UV, MS, IR and NMR it was clear that compound **2** is an isomer of compound **1**.

Table 1: ^1H and ^{13}C NMR for Compounds 1 and 2

1				**2**		
Position	δC	δH		δC	δH	
C=O	169.320					
α	125.392	7.72308	1Hd			
β	145.980	7.71379	1Hd			
1	105.132					
2	129.143	7.61050	1Hs	143.812		
3	108.933			148.005	7.05446	1Ht
4	145.229	6.66744	1Hd	24.412	1.28688	2Hd
5	145.581	6.69722	1Hd	143.812	7.61012	1Hd
6	146.271	5.08174	OH (brs)	128.241	7.61843	1Hd
7				125.582	4.84209	OH (brs)
8				128.241	7.05446	1Hs
9				129.037		
10				130.442		
1^l	128.760			128.241		
2^l	131.027	7.61050	2Hd	130.442	7.62494	1Hd
3^l	110.328	7.05838	1Hd	125.582	7.63279	1Hd
4^l	166.622	5.32788	OH (brs)			
5^l	110.328	7.05838	1Hd	125.582	7.63279	1Hd
6^l	131.027	7.61050	2Hd	130.442	7.62494	1Hd
7^l	125.392	4.58279	1Hd	143.812	4.22288	1Hd
8^l	129.149	4.46730	1Hm	148.006	4.22672	1Hm
9^l	23.640	1.27960	3Hd	24.472	1.28688	3Hd

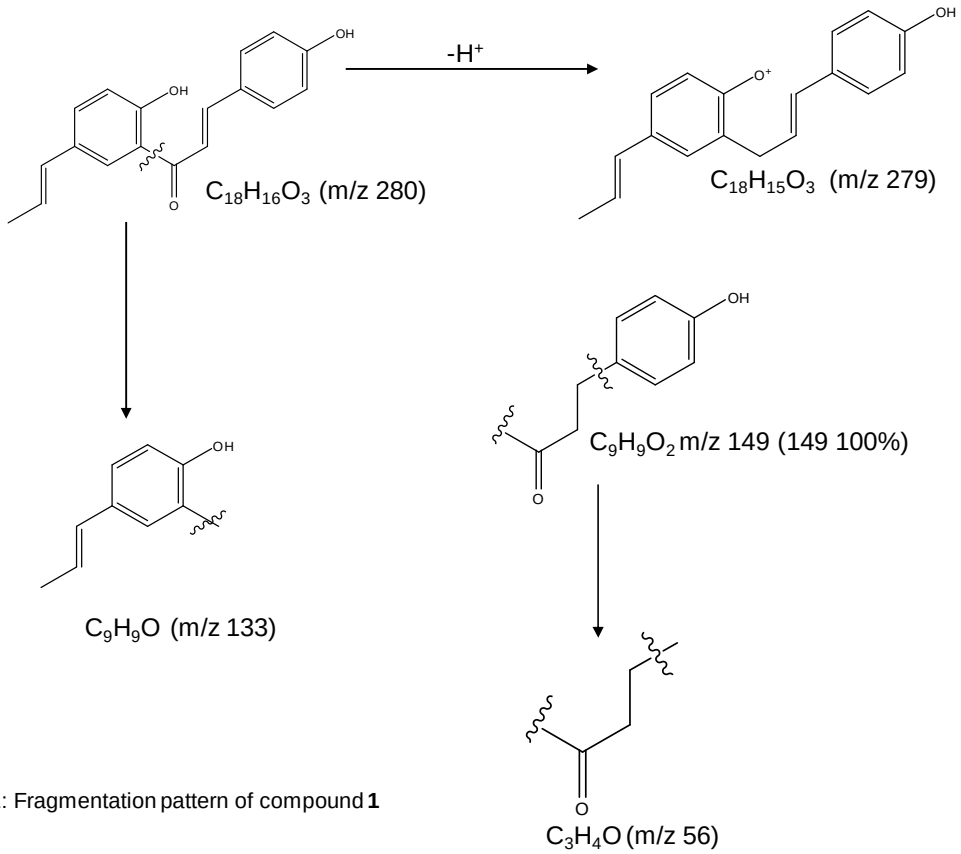


Fig 1: Fragmentation pattern of compound **1**

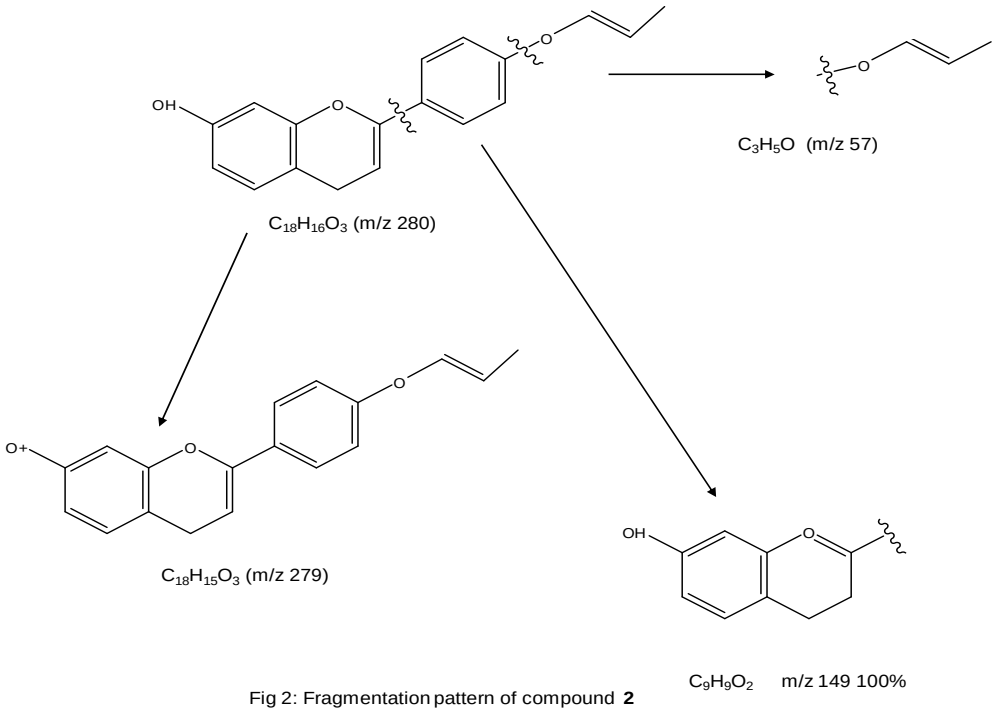
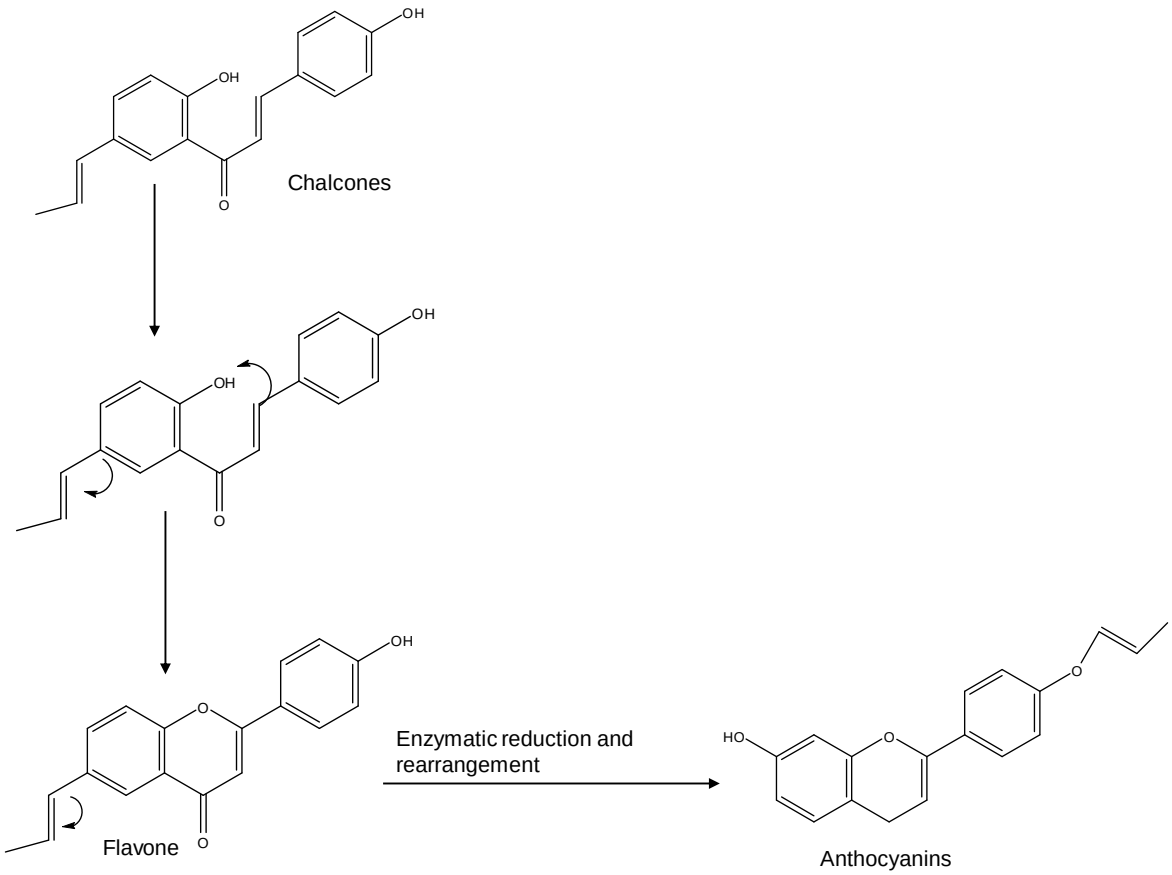


Fig 2: Fragmentation pattern of compound **2**



Scheme 1: Biosynthesis of Anthocyanins from Chalcones

The conjugation of the electrons of the heterocyclic ring within the aromatic system may be responsible for the yellow color of anthocyanidine. The presence of these phenolic compounds indicates that this plant may be anti-microbial agent since phenols and phenolic compounds are extensively used in disinfections and remain the standard with which other bactericides are compared [10]. The phenolic compounds in *B. ferruginea* may be responsible for the therapeutic, antiseptic, antifungal or bactericide, as well as anti-viral and anti-tumor activities of *B. ferruginea* [5,7]. The phenolic compounds undergoes oxidation and form phenolate ions or quinone. The phenolate ions are able to scavange and trap organisms [10]. The bioactive phenolic compounds act as radical scavengers and singlet oxygen quenchers. They react with peroxyl radicals and thus bringing about the termination of the radical reaction generated within the system. The antioxidant, anti-inflammatory and anti-carcinogenic activities of *B. ferruginea* may be attributed to the presence of the flavonoids, chalcones, flavones and anthocyanidines obtained from the plant. The propose mechanism of action range from scavenging reactive oxygen species to inhibiting enzymes responsible for cancer promotion and progression. They have the ability to block the release of the enzymes responsible for pain and inflammation, this supports the use of *B. ferruginea* in herbal medicine for the treatment of arthritis, elephtiasis and muscular dystrophy. The presence of α – β – unsaturated keto group in chalcones is found to be responsible for their biological activity [11-13]. In the present life styles, where stress conditions are common, leading to excess production of free radicals, these natural products will prove a support to our biological system to sustain and balance metabolism. These findings supported the use of *Bridelia ferruginea* in phytomedicine as antioxidant and anti-inflammatory agent for disease treatment and prevention in Nigeria. The isolated compounds from *Bridelia ferruginea* can be use by pharmaceutical firms for drug formulation.

Acknowledgements
The authors are grateful to Dr. Nkechi Ibisi for her assistance in obtaining NMR spectra and Mass spectra from China.

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