Sudan University of Science and Technology

The Graduate College

Spectral Study on the Major Flavonoid from

Leptadenia heterophylla

دراسة طيفية للفلافونيد الرئيس في نبات عرق المحبة

A Thesis Submitted in Partial Fulfillment of the Requirements of the M.Sc. Degree in Chemistry

By

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(B.Sc. Honors chemistry)

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(وَقُل رَّبِّ زِدْنِي عِلْمًا)

(طه : 114)

Dedication

dedicated to

Soul of my father,

My lovely mother

And

Sisters

Acknowledgements

Thanks to Almighty Allah for giving me strength and health to accomplish this work. I would like to express my gratitude to my supervisor Prof, Mohammed Abd Elkarim for continuous supervision, valuable suggestions and advice, his kind help enabled me to achieve my research goals.

Thanks are also extended to the family of Sudan Institute for Natural Sciences for their assistance and support.

Many thanks to my family, friends and colleagues at the research laboratory for their help and encouragement.

Also I would like to thank Miss Valentina, Mrs. Tamador and Miss Walaa, of the Central Lab., Khartoum University for the UV and IR spectral measurements.

Abstract

The flavonoids of the roots of *Leptadenia heterophylla* were investigated. Extraction of roots with 95% ethanol gave a crude extract. Phytochemical screening revealed the presence of flavonoids, glycosides, steroids and absence of alkaloids.

The crude extract was fractionated by paper chromatography irrigated by 40% acetic acid. Only one major component was detected under UV light. After the usual workup, the major component- compound I - was isolated. The structure of the isolate was partially deduced on the basis of its UV and IR spectra. The spectral data revealed that compound I is either: a 5-hydroxyflavanone or a 5-hydroxydihydrochalcone.

الخلاصة

لقد تمت دراسة الفلافونيد الرئيس فى نبات عرق المحبة , حيث استخلصت الجزور بالاثانول (95%). اجرى مسح فيتوكيميائي للمستخلص الكحولي والذي اوضح وجود الفلافونيدات بجانب الجلايكوسيدات والاسترويدات إلا أن القلويدات لم تكن بالنبات.

تمت تنقية المستخلص الخام بكروموتوغرافيا الورق التي استخدم فيها حمض الخليك بتركيز 40% كمذيب. تحت الاشعة فوق البنفسجية اتضح وجود فلافونيد رئيس وحيد .

تم فصل هذا الفلافونيد نقياً ووضح التركيب جزئياً بمطيافية الاشعة فوق البنفسجية ومطيافية الاشعة تحت الحمراء , حيث اتضح أنه يمكن أن يكون :

(5-hydroxyflavanone) أو (5-hydroxydihydrochalcone).

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1-Introduction

Chapter Two

2-Materials and Methods

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Chapter One

Introduction

1-Introduction

1.1- General approach

Flavonoids are characterized by a benzopyran skeleton. In this class of plant phenolics two phenyl rings are joined by a 3 carbon bridge. This group of natural products are isolated from a wide variety of plants, and are responsible for much of the colouring found in vascular plants. A single plant may contain dozens of different flavonoids, and the distribution of flavonoids within a plant family can yield useful classifying information about that family1.

Many flavonoids are easily recognised as flower pigments in most angiosperm families (flowering plants). However, their occurence is not restricted to flowers but include all parts of the plant2. They also play a pivotal role in plant development, growth and defence3.

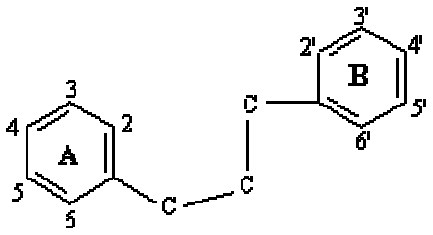
Most flavonoids possess antioxidant, anti-inflammatory, and antiviral properties. They also help to maintain the health of small blood vessels and connective tissue, and some are under study as possible treatments of cancer3.

Flavonoids occur naturally in fruit, vegetables, and beverages such as tea and over 4000 structurally unique flavonoids have been identified in plant sources4 For example the flavonol quercetin and the flavone apigenin are found in many fruits and vegetables, including onions, apples, broccoli, and berries. Naringenin is a

citrus flavanone. Catechin and other catechins are abundant in green tea. Cyanidin and other anthocyanidins are largely responsible for the deep colors of berries and grapes. Genistein is an isoflavone found predominantly in legumes. The flavonoid consumed most, in general, is quercetin, and the richest sources of flavonoids consumed in general are tea, onions, and apples.

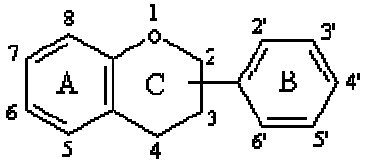
Research in the field of flavonoids has rapidly increased. The major actions of flavonoids are those against cardiovascular diseases, ulcers, viruses, inflammation, osteoporosis, diarrhea and arthritis. Brief description about the disease causing effect of free radicals was given and ways by which flavonoids neutralize free radicals has also been mentioned[4]. Plants containing flavonoids have historically been used in traditional eastern medicine[4]. Flavonoids exhibit a wide range of biological activities, and currently are of particular interest as potential anticancer, antiallergic, antithrombotic antibacterial, antifungal and antitumoral agents[4.]

The flavonoids are polyphenolic compounds possessing 15 carbon atoms; two benzene rings joined by a linear three carbon chain[5].



The above skeleton can be represented as the C_6 - C_3 - C_6 system[5].

The basic skeleton of the majority of flavonoids consist of two fused six-membered rings (an aromatic A-ring and a heterocyclic C ring) connected to an aromatic B-ring as shown below:



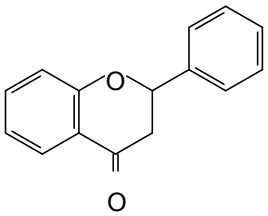
Depending on the position of the linkage of the aromatic ring to the benzopyrano (chromano) moiety, this group of natural products may be divided into three classes:

-Flavonoids (1)

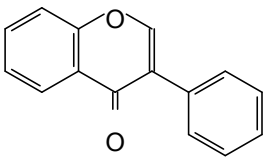
-Isoflavonoids (2)

-Neoflavonoids (3)

These groups usually share a common chalcone precursor, and therefore are biogenetically and structurally related[2].



(1)



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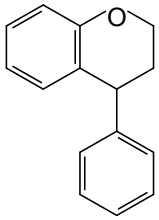
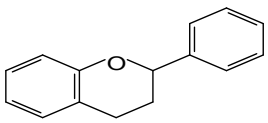


(3)

Depending on the degree of oxidation and saturation present in the heterocyclic C-ring, flavonoids may be divided into: flavan(4),flavanone(5) , flavones(6) and flavonols(7)[2].



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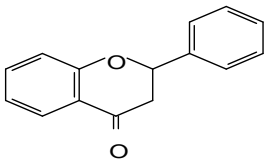

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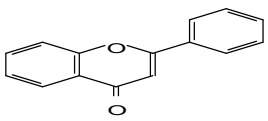

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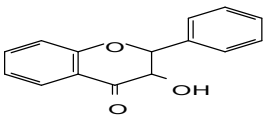
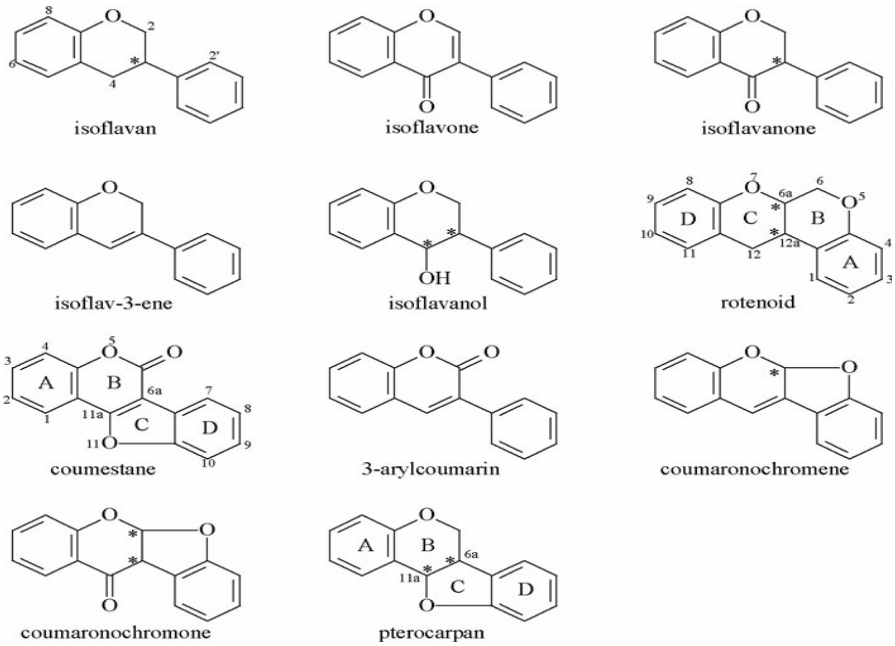


(7)

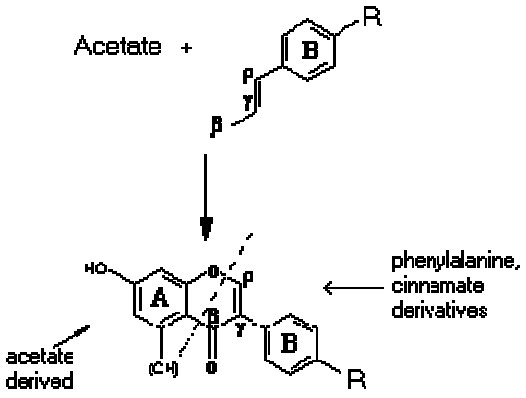
1.2-Isoflavonoids

Isoflavonoids constitute a distinctive subclass of the flavonoids. These compounds possess a 3-phenylchroman skeleton that is biogenetically derived by 1,2-arylmigration in a 2-phenylchroman precursor. Despite their limited distribution in the plant kingdom, isoflavonoids are remarkably diverse as far as structural variations are concerned. This arises not only from the number and complexity of substituents on the basic 3-phenylchroman system, but also from the different oxidation levels`and presence of additional heterocyclic rings[2]. Isoflavonoids are subdivided into isoflavan ,isoflavone , isoflavanone , isoflavan-3-ene , isoflavanol , rotenoid , coumestane , 3-aryl coumarin , coumaronochromine ,coummaronochromone , pterocarpan .



When compared with other flavonoids, isoflavonoids have a rather limited taxonomic distribution, mainly within the *Leguminosae*. Most of our knowledge about the biosynthesis of isoflavonoids originates from studies with radioactive isotopes, by feeding labelled ^{13}C cinnamates[5].

All isoflavonoids are colourless. It has been established that acetate gives rise to ring A and that phenylalamine, cinnamate and cinnamate derivatives are incorporated into ring B and C-2, -3, and -4 of the heterocyclic ring[5].

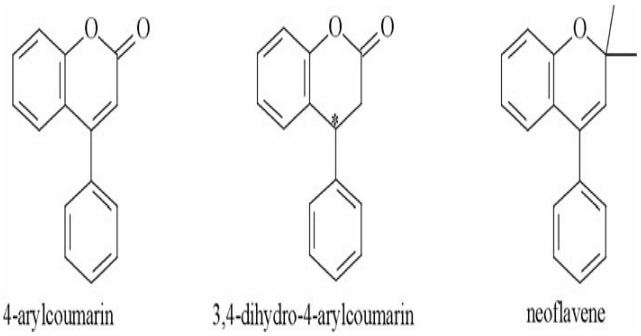


Since chalcones and flavanones are efficient precursors of isoflavonoids, the required aryl migration of ring B from the former 2 or beta position to the 3 or alpha position of the phenylpropanoid precursor must take place after formation of the basic C_{15} skeleton[5].

1.3- Neoflavonoids

Neoflavonoids are structurally and biogenetically closely related to the flavonoids and the isoflavonoids and comprise the 4-arylcoumarins (4-aryl-2*H*-1- benzopyran-2-ones) , 3,4-

dihydro-4-arylcoumarins , and neoflavene .

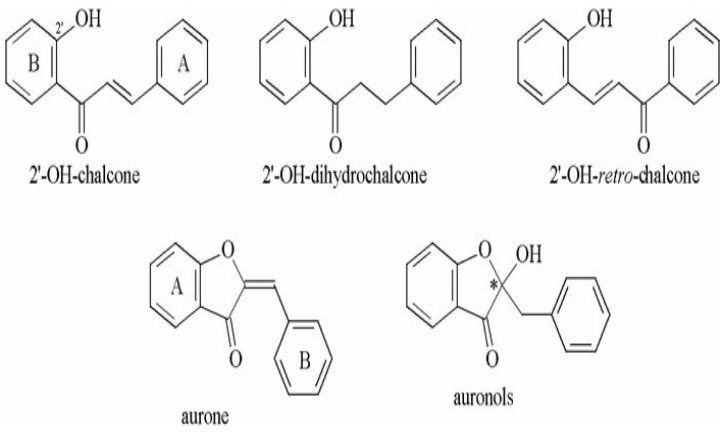


(2)

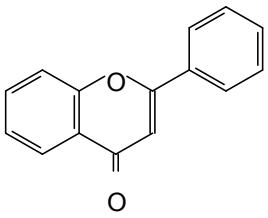
The isoflavonoids and neoflavonoids can be regarded as abnormal flvonoids[5].

1.4- Minor flavonoids

Chalcones and aurones also contain a C_6-C_3-C_6 backbone and are considered to be minor flavonoids. These groups of plant phenolics include: the 2′-hydroychalcones, 2′-OH-dihydrochalcones, 2′-OH-*retro*-chalcone, aurones (2-benzylidenecoumaranone), and auronols[2].



1.5- The flavones



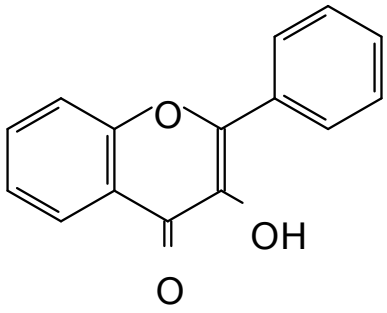
Examples of flavones include the widespread apigenin and luteolin. Isoflavones are isomers of flavones in which the B ring of the flavonoid nucleus is attached to C_3[6].

The A –ring of the great majority of flavones is derived from phloroglucinol and the B- ring is oxygenated in the 4`,or 3`,4`-or 3`,4`,5`,positions as expected from their established acetate-shikimate biosynthetic origin[6] .

Flavones are mainly found in cereals and herbs. In the West, the estimated daily intake of flavones is in the range 20–50 mg per day[7]. In recent years, scientific and public interest in flavones has

grown enormously due to their putative beneficial effects against atherosclerosis, osteoporosis, diabetes mellitus and certain cancers[8]. Flavones intake in the form of dietary supplements and plant extracts has been steadily increasing.Flavones have effects on CYP (P450) activity[9,10.]

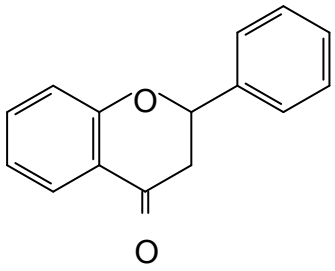
1.6-Flavonols



Flavanols are similar to flavones they share the same backbone, but flavonols are characterized by a 3-OH function. In addition to having antioxidant qualities, research indicates that flavanols have other positive influences on vascular health, such as lowering blood pressure and improving blood flow to the brain and heart, making blood platelets less sticky and able to clot, and lowering cholesterol[11].

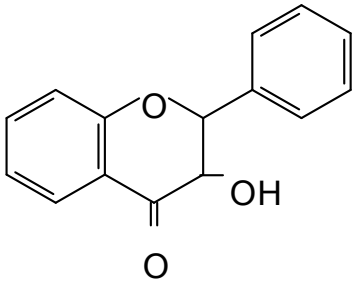
Since flavonols are simply flavones in which the 3-position is substituted by a hydroxyl ;both classes of pigments have so far been considered together[6] .

1.7-Flavanones



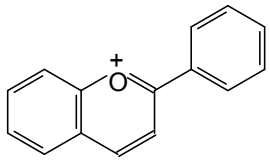
Flavanones are described as a : 2-phenyl-benzopyran-4-one.The parent compound is not known to be naturally occurring; The simplest plant flavanone has a hydroxyl group at position7.Fllavanones are isomeric with chalcones from which they can be obtained synthetically and from which they arise biosynthetiacally .Flavanones have a center of asymmetry at C-2 so that naturally occurring members are often optically active. Flavanones have attracted interest since they obligate intermediates in flavonoid biosynthesis .They can be dehydrogenated to yield flavones or can undergo hydroxylation at position -3 to yield dihydroflavonols (3- hydroxyl flavanoids)[1].

1.8-Dihydroflavonols



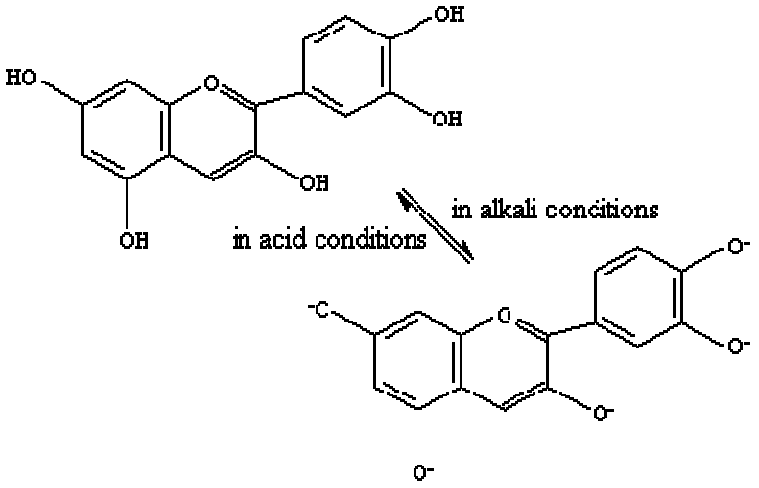
The dihydroflavonols are 2-phenyl-3-hydroxybenzo-pyran-4-one .7-Hydroxy-dihydroflavonol is the simplest known naturally occurring member of the series. Dihydroflavonols have two asymmetric carbons, C-2 and C-3, the streochemical implications of which will be mentioned in a separate section .Dihydroflavonnols are interesting compounds, since they obligatediate in flavonoid biosynthesis. Dihydroflavonls, in turn can yields several other types; dehydrogenation yields flavonols Reduction of the carbonyl function affords flavan -3,4-diols, enolization and oxidation yields anthocyanidins[1] .

1.9- Anthocyanins

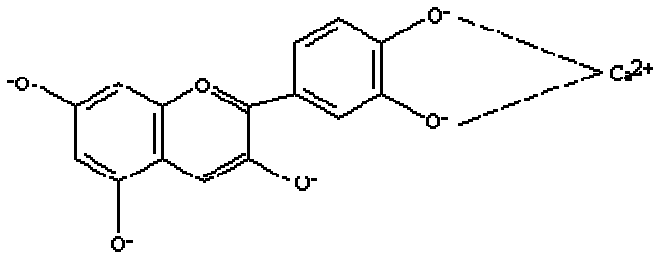


Anthocyanidin represent an extended conjugation made up of the aglycone of the glycoside anthocyanins. Next to chlorophyll, anthocyanins are the most important group of plant pigments visible to the human eye.

These plant phenolics constitute a large family of differently coloured compounds and occur in countless mixtures in practically all parts of most higher plants. They are of great economic importance as fruit pigments and thus are used to colour fruit juices, wine and some beverages[5]. The anthocyanidins in hydrangea, colours it red in acid soil and blue in alkali soil[3].

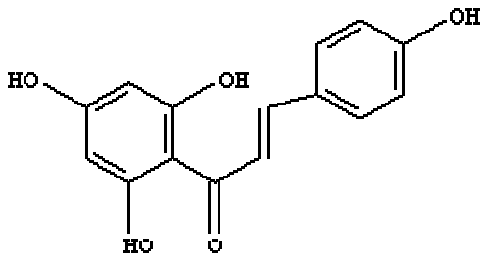


Anthocyanins could chelate with metal ions like Ca^{2+} and Mg^{2+} under alkali conditions[5].This extends the conjugation as shown below:



1.10-Chalcones

As shown below chalcone is derived from three acetates and cinnamic acid as shown below[5]. Chalcones are open chain flavonoids in which the two aromatic rings are joined by a three carbon chain[5].



Chalcones can be considered to be derivatives of phenyl styryl ketones .Naturally occurring chalcones are all hydroxylated to a greater or lesser extent .The parent compound chalcone itself is not known as a natural product[5] .

A common chalcone is butein - 2-,4-,3,4-tetra-hydroxychalcone. Butein occurs free in the wood or bark of several trees , e.g. Acacia, Adenanthera and Rhus[5].

1.11- Synthesis of flavonoids

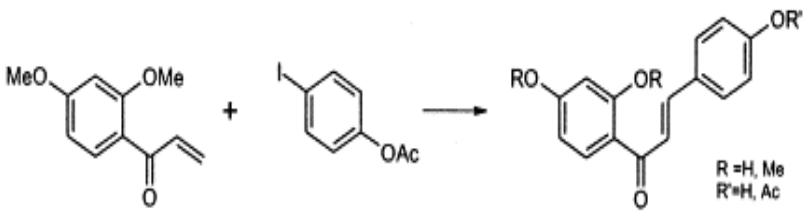
The synthesis of flavonoids have been subject of great number of studies. Although there are several types of skeletons (flavones, isoflavonoid, aurones, etc.), it is the the flavones whose synthesis has been more widely studied.The most used strategy is the reaction of substituted acetophenones with corresponding substituted benzaldehydes either in basic or acidic media[12].

In the field of microwave – assisted organic reactions, flavonoids have also been studied. However, in this field there was a lack of data for the two component reaction of acetophenones and benzaldehydes. Although synthesis of chalcones by reaction of

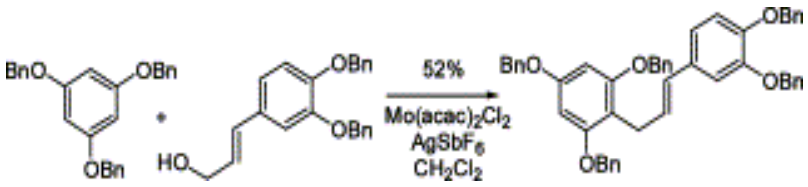
these components[13] were reported, all of the acetophenones studied, lacked the 2-OH-substituent that could allow the subsequent cyclization to close the pyrane ring present in flavanones. Otherwise there have been reported cyclizations of chalcones to 3-substituted-flavanones[14] , diphenyl - β - diketones to flavones[15] and 2-aminochalcones to 2-aryl-1,2,3,4-tetrahydro-4-quinolones[16]. But it seemed that nobody had studied (or at least reported) the above mentioned approach of two component addition-cyclization without the addition of a second molecule of aldehyde to position 3 of the flavanone[17].

Among the wide variety of reaction conditions for the classical reaction, Chang's reagent (SiO_2/H_3BO_3/piperidine/DMF) was attempted[18]. It was observed that shorter irradiation time lead to better yield[19].

Many flavonoids have been synthesised following a new proposed method based on the use of the Heck reaction. The key step involves the coupling of an aryl vinyl ketone with an aryl iodide. This procedure affords the flavonoid moiety in a single step[20].



The above mentioned method deals with the formal total synthesis of flavonoids bearing the hydroxylation pattern of the catechin series based on an access to the fully functionalized skeleton via the alkylation of phloroglucinol tribenzyl ether by 3,4-dibenzyloxycinnamyl alcohol. This reaction was revealed to be most successful when catalyzed by the $Mo(acac)_2(SbF_6)_2$ complex. In addition, the underlying concepts to the different ways that can be used in this C_6–C_3+C_6 strategy are discussed[21].

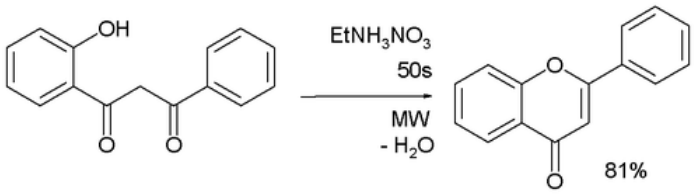


Interest in the biological properties of flavones has resulted in intense synthetic efforts towards the synthesis of various flavones. There are a number of methods reported for the synthesis of flavones.

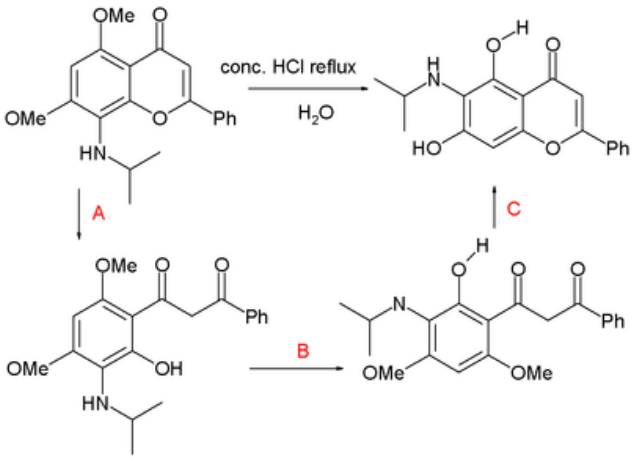
Several methods exist for the synthesis of flavones:

- Allan-Robinson reaction
- Baker-Venkataraman rearrangement
- Algar-Flynn-Oyamada reaction

The basic skeleton of flavonoids is also available via dehydrative cyclization of certain 1,3-diaryl diketones. The effect of an ionic liquid solvent and microwave irradiation on the yield of this process was studied[22].



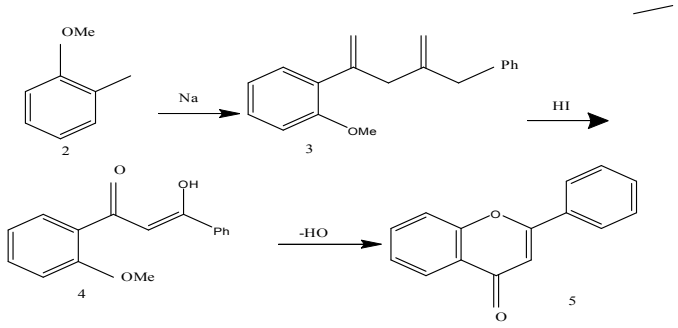
An important tool in structure elucidation of flavonoids is the Wessely-Moser rearrangement[23]. It involves the conversion of 5,7,8-trimethoxyflavone into 5,6,7-trihydroxyflavone on hydrolysis of the methoxy groups to phenol groups. It also has synthetic potential for example[24]:



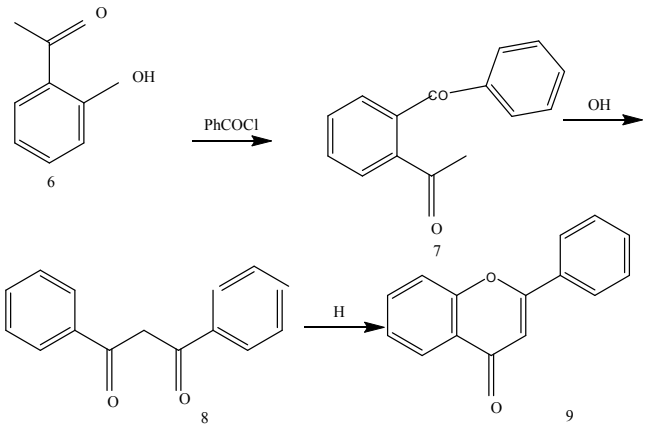
Such rearrangement takes place in several steps: (**A)** ring opening to the diketone, (**B)** bond rotation with formation of a favourable acetylacetone (C) hydrolysis of two methoxy groups and ring closure[25].

A general method for synthesizing flavones is the Von-Konstanecki method which involves a reaction of o-methoxybenzoate and acetophenone in the presence of sodium to form (3). This is followed by treatment of (3) with an acid to

form compound (**4**) followed by elimination of water in order to form the flavones (5)[26].



The most convenient route to the synthesis of flavones is the Baker-Venkataraman approach. In this reaction, 2-hydroxyacetophenone is converted to ester, which then undergoes rearrangement by intramolecular Claisen condensation in the presence of potassium hydroxide and pyridine to afford 1,3-diketone which is then cyclised to flavone under rather harsh conditions either by treatment with concentrated sulfuric acid or heating with glacial acetic acid[27].

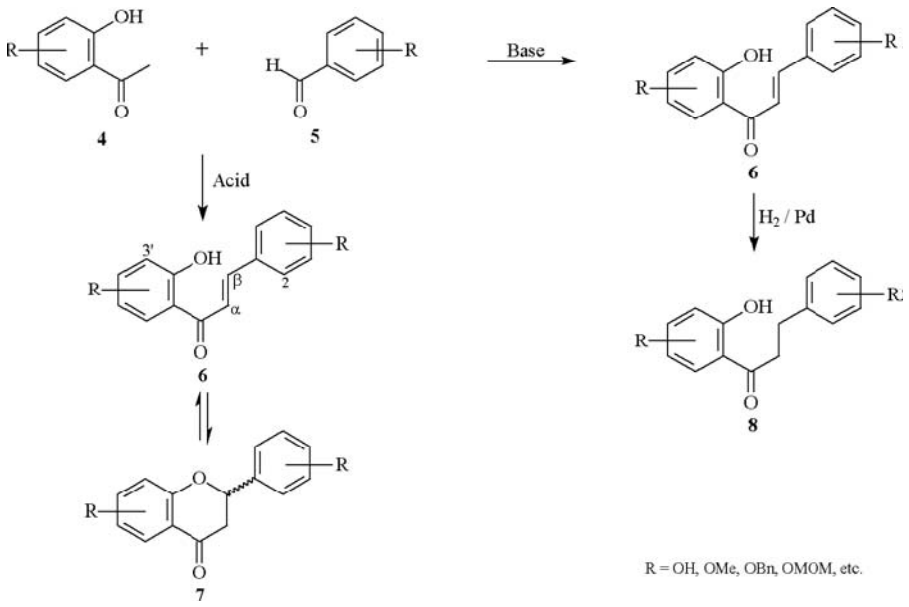


1.12- Chalcones

Chalcones and dihydrochalcones are considered to be the primary C6-C3-C6 precursors and constitute important intermediates in the synthesis of flavonoids[2].

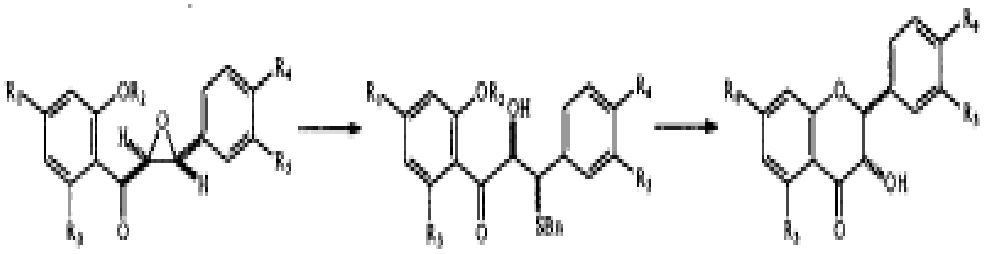
Chalcones are readily accessible via two well-established routes comprising a base-catalyzed aldol condensation or acid-mediated aldolization of 2-hydroxyacetophenones and benzaldehydes[15] .The base-catalyzed aldol condensation is usually the preferred route toward chalcone formation, since under acidic conditions cyclization of the ensuing chalcone leads to formation of corresponding racemic flavanones . Dihydrochalcones are generally obtained via reduction (H_2/Pd) of the preceding chalcones[2].

Conventional base-catalyzed aldol condensation usually employs NaOH or KOH, but other bases like NaH have also been utilized to produce chalcones in up to 89 % yield. (93) These compounds can also be obtained in high yields (75 - 96 %) by Lewis acid catalysis, e.g. borontrifluoride-etherate[28].



1.13- *Trans*- and *cis*-dihydroflavonols

Epoxidation of a series of poly-oxygenated chalcones with H_2O_2 in the presence of poly-α-aminoacids yields chiral aromatic oxygenated oxiranes in moderate to high optical yields. Lewis acid-catalysed phenylmethanethiol ring opening of the epoxide functionality and subsequent formation of the pyranone heterocycle, afforded *trans*- and *cis*-dihydroflavonols in moderate to high enantiomeric excess and yield[29].



R1, R3, R4, R5 = H, OMe; R2 = H, MOM

1.14-Isolation and identification of flvonoids

Flavonoids are present inside the cells or on the surfaces of different plant organs. They differ in the saturation of the heteroatomic ring C, in the placement of the aromatic ring B at the positions 2,3 or 4 of ring C, and in the overall hydroxylation patterns . The flavonoids may be modified by hydroxylation, methoxylation, or O-glycosylation of hydroxyl groups as well as C-glycosylation directly to carbon atom of the flavonoid skeleton.

Alkyl groups (often prenyls) may be covalently attached to the flavonoid moieties, and sometimes additional rings are condensed to the basic skeleton of the flavonoid core[2]

Different plant families have characteristic patterns of flavonoids and their conjugates. All these compounds play important biochemical and physiological roles in the various cell types or organs (seed, root, green part, fruit) where they accumulate. Different classes of flavonoids and their conjugates have numerous functions during the interactions of plant with the environment, both in biotic and abiotic stress conditions[18]. Additionally, flavonoid conjugates, because of their common presence in plants, are important components of human and animal diet. Due to the different biological activities of plant secondary metabolites, their regular consumption may have serious consequences for health, both positive and negative[6.] For the mentioned reasons, methods for the efficient and reproducible

analysis of flavonoids play a crucial role in research conducted in different fields of the biological and medical sciences[2].

The structural characterization of flavonoids and their conjugates isolated from plant material, as single compounds or as part of mixtures of structurally similar natural products, create some problems due to the presence of isomeric forms of flavonoid aglycones and their patterns of glycosylation. A number of analytical methods are used for the characterization of flavonoids. In many cases, nuclear magnetic resonance (NMR) analyses (^{1}H and ^{13}C) are necessary for the unambiguous identification of unknown compounds; other instrumental methods (mass spectrometry, UV and IR spectrophotometry) applied for the identification of organic compounds fail to provide the information necessary to answer all the structural questions. Utilization of standards during analyses and comparison of retention times as well as spectral properties, especially when compounds are present in a mixture, is critical. An important area of research on flavonoids is the identification of their metabolites in animal tissues and body fluids (urine, blood, spinal fluid). For this, investigators have to deal with different modifications of the flavonoid moieties, modifications often not found in plant tissues[30.]

1.15- Biological activity

It is claimed that some flavonoids are believed to inhibit certain enzymes in biological systems, such as lipoxygenase, cyclo-oxygenase, mono-oxygenase, xanthine oxidase, mitochondrial succinoxidase, reduced nicotinamide-adenine dinucleotide (NADH) oxidase, phospholipase A2, topoisomerases and protein kinase[31,32].In total the pharmacological effects of flavonoids are mainly ascribed to their antioxidant activities[33-36],as radical scavengers, reductants and metal chelators[37].but their non-antioxidant functions are also believed to make a contribution. The latter include interactions with different enzymes, inhibition of calcium ion reflux into cells and regulation of cell signalling and gene expression[38].As a reductant, the flavonoids are oxidized in order to reduce other biological molecules. Acting as a metal chelating agent they reduce the capacity of a metal to produce free radicals. These effects can, however, not be assigned exclusively to the flavonoids since other biological components may directly contribute or enhance them[38].

Flavonoids of fruits and vegetables have proven to be powerful free radical scavengers both *in vitro* (neutralizing synthetic free radicals) and *in vivo* (neutralizing physiologically relevant peroxyl radicals, hydroxyl radicals and superoxides)[39]. Catechins, for instance were shown to scavenge radicals *via* electron transfer or by acting as a hydrogen donor.

Green leafy vegetables and citrus fruits as well as berries all have high potential of antioxidant activities but these potentials differ vastly even within the same variety. Different cultivation sites, climates, stages of maturity and sample preparation and extraction procedures are believed to contribute to this observation[40].

It was claimed that the consumption of flavonoid-containing foods is inversely related to coronary heart disease[41] and genotoxic activity[42]. Atherosclerosis is caused by cholesterol-loaded macrophages, which originates from the internal oxidation of low-density lipoproteins (LDL).

Dietary consumption of flavonoids causes an increase in antioxidant capacity in cells which result in an inhibition of oxidation of LDL thus preventing atherosclerosis to a certain extent. ***Glycyrrhiza glabra***, the licorice plant, generally used as sweetening or flavouring agent, is one example which reduces LDL oxidation. This corresponds with the well-known French paradox in which the population of southern France suffers a low cardiovascular mortality in spite of having a diet which is high in saturated fats but is accompanied with moderate daily consumption of flavonoid-rich diet[41].

1.16- Specific Examples of Flavonoids

Individual flavonoids are categorized in a variety of ways, sometimes overlapping categories. Although they are all structurally related, they do different jobs. Here are the most well-known flavonoids and some of their uses:

-**Anthocyanins** — good for circulation, vision and brain function. Abundant in acai berries, goji berries, mangosteen and noni.

-**Hesperidin** — an antiviral flavonoid and effective histamine-blocker; works in tandem with vitamin C.

-**Rutin** — good for circulation, younger-looking skin and a host of other benefits; works in tandem with vitamin C.

-**Quercetin** — a powerful antioxidant, anti-inflammatory, circulation booster: works in tandem with vitamin C.

-**Curcumin** — antioxidant, anti-inflammatory and anti-carcinogenic nutrient; source of the spice turmeric[43, 44].

1.17- Functions of flavonoids in plants

Some flavonoids have inhibitory activity against organisms that cause plant disease eg. ***Fusarium oxysporum***[44]

Flavonoids are widely distributed in plants fulfilling many functions.They are the most important plant pigments for flower coloration producing yellow or red/blue pigmentation in petals designed to attract pollinator animals.

Flavonoids secreted by the root of their host plant help *Rhizobia* in the infection stage of their symbiotic relationship with legumes like peas, beans, clover, and soy. Rhizobia living in soil are able to sense the flavonoids and this triggers the secretion of nod factors, which in turn are recognized by the host plant and can lead to root hair deformation and several cellular responses such as ion fluxes and the formation of a root nodule[4].

1.18- *Leptadenia* species

Mohamed *et.al*. studied[45] the aqueous extract of the aerial parts of ***Leptadenia pyrotechnica*** and found that it contains five polyphenolic compounds , a major component being a quercetin glucoside.

Amal *et.al.* investigated[46] the antitumour potential of ***Leptadenia pyrotechnica*** and concluded that the antitumour activity is due to the flavonoids present in this species.However, some

Leptadenia pyrotechnica

Leptadenia pyrotechnica flower

Leptadenia pyrotechnica seed pods

alkaloids were also characterized. Three terpenes- phytol, squalene and taraxerol- and five sterols- cholesterol,co- mpasterol, stigmasterol, β-sitosterol and fucosterol were isolated from ***Leptadenia pyrotechnica***[47].

Leptadenia pyrotechnica was estimated[48] for its antifungal activity against some pathogenic fungi. Extracts in different organic solvents were used for disc diffusion assay. The hexane and ethyl acetate extracts have shown maximum inhibition zones against ***A.niger***. Methanolic extract showed maximum inhibition against ***F. oxysporium*** and ***A.flavus***, while aqueous extract exhibited significant activity against ***F.monilifermis***.

The aqeous ethanolic crude extract of *Leptadenia pyrotechnica* aerial parts along with its ethyl acetate ,n-butanol and water partition fractions were evaluated[49] for their antio,xidant capacity,Polyphenol content, aintiflammatory and anti-cancer properties. The ethyl acetate fraction showed the maximum polyphenol content and best antioxidant potential.

Leptadenia spacuta is used in ethnomedicine for the treatment of uterine cance[50].Also ***Leptadenia hastate*** was shown [51] to possess antitumour activity. The activity was attributed to some dietary terpenes.

Leptadenia hastate

Leptadenia hastate flower

Baheti and Awati[52] investigated the possible anti-asthmatic activity of ***Leptadenia reticulate*** and concluded that the hydro alcohol extract of the leaves exhibited significant anti-asthmatic activity. The authors attributed this activity to β-sitosterol which detected by HPLC in the saponin fraction of the faqueous extract.

Leptadenia reticulate

The chloroform extract of *Leptadenia reticulate* was evaluated [53] *in vitro* for aphrodisiac potential. The extract showed significant aphrodisiac activity in model animals at different doses (50,100 and 250mg/Kg).

1.19-*Leptadenia heterophylla*

Taxonomy:
Kingdom: Plantae
Phylum: Magnoliophyta
Class: Magnoliopsida
Order: Gentianales
Family: Asclepiadaceae
Genus: Leptadenia

Leptadenia heterophylla

Leptadenia heterophylla

1.20-Aim of this study

This study was aimed to:

i) Extraction of plant phenolics
ii) Isolation of the major flavonoid from targeted species.
iii) Conducting UV and IR studies on the isolated components.

Chapter Two

Materials and Methods

2-Materials and Methods

Analytical grade reagents were used. The IR spectra were run on FTIR-8400S, Fourier Transform Infrared Spectrophotometer. The UV Spectra were recorded on a Perkin-Elmer UV-1800 Spectrophotometer.

2.1-Materials

2.1.1-Collection of plant material

Leptadenia heterophylla was collected from Damazin-Sudan and authenticated by the Aromatic Plants Research Institute.

2.2-Methods

2.2.1-Preparation of test reagents for phytochemical screening

2.2.1.1-Flavonoid test reagents

i.Aluminum chloride solution

(1g) Aluminum chloride was dissolved in (100ml) methanol

ii.Pototassium hydroxide solution

(1g) Potassium hydroxide was dissolved in (100ml) water

iii.Ferric choloride solution

(0.5g) ferric chloride was dissolved in (100ml) 97%methanol.

2.2.1.2- Alkaloid test reagents

Modiffied Dragendeoffs reagents

i. Stock Solution (A)

(0.5g) Bismuth nitrate was dissolved in (10ml) acetic acid and (40ml) of water was added.

ii. Stock Solution (B)

(8g) Potassium Iodide was dissolved in (20ml) water.

When testing for alkaloids, (5ml) of stock solution (A) is mixed with (5ml) of stock solution (B) 20ml of acetic acid and (100ml) water were added.

2.2.2- Plant extract

Powderd air-dried roots of *Leptadenia heterophylla* were macerated at room temperature with 95% ethanol (5L) for 48hr.. The solvent was evaporated under reduced pressure and residue was used for the following tests.

2.2.3- Phytochemical screening

The crude ethanolic extract was screened for steroids, flavonoids, alkaloids and glycosides.

2.2.3.1- Test for steroids

Part of the crude extract was stirred with petroleum ether to remove most of the colouring matter. The residue was extracted with (20ml) chloroform and dehydrated over anhydrous sodium sulphate.

(5ml) Portion of the solution was mixed with (0.5ml) acetic anhydride, followed by two drops of concentrated sulphuric acid .Development of a green colour was taken as a positive test for steroids .

2.2.3.2- Test for alkaloids

(5ml) of 2N hydrochloric acid were added to the crude extract and the solution was heated with stirring in a water bath for 10 minutes. The cooled solution was filtered .To portion (5ml) of this solution; few drops of Dragendroffs reagent were added .No precipitate was formed indicating absence of alkaloids.

2.2.3.3- Test for flavonoids

Part of the crude extract was defatted by extraction with petroleum ether. The defalted residue was dissolved in (30ml) 95%ethanol and filtered. The filtrate was used for the following tests:

i. To (3ml) of filterate, few drops of 1% methanolic aluminium chloride were added. Formation of yellow colour indicated the presence of flavonoids.

ii. To (3ml) of filtrate, few drop ofpotassium hydroxide solution were added, a dark yellow colour indicated the presence of flavonoids.

iii. To (3ml) of filtrate,few drops of ferric chloride solution were added. Development of a blue colouration was taken as a positive test for flavonoids.

2.2.3.4- Test for glycosides

Part of the powdered air-dried plant was vigorously shaken in a test tube with water. The presence of a froth that persisted for one hour indicated the existence of glycosides.

2.2.4-Isolation of flavonoids

Part of the crude extract (ca.0.5g) was dissolved in ethanol (2ml) and applied as a narrow zone on Whatman No. 3 paper sheets. The sheets were irrigated with 40% acetic acid. The zones containing flavonoids were located under UV lamp. The major component was eluted from paper by ethanol. Removal of the solvent under reduced pressure gave compound I.

2.2.5-UV shift reagents

i. Sodium Methoxide Stock Solution

Freshly cut metallic sodium (2.5g) was added cautiously in small portions to dry spectroscopic methanol (100ml). The solution was stored in a glass container with a tightly fitting plastic stopper.

ii. Aluminium chloride stock solution

(5g) of fresh anhydrous aluminium chloride were added cautiously to spectroscopic methanol (100ml).

iii. Hydrochloric acid stock solution

Concentrated hydrochloric acid (50ml) was mixed with water (100ml) and stored in a glass stopper bottle.

iv. Boric acid

Anhydrous powdered reagent grade H_3BO_3 was used.

v. Sodium acetate

Anhydrous powdered reagent grade NaOAC was used.

2.2.6- The UV spectrum in presence of UV shift reagents

2.2.6.1- The UV spectrum in presence of sodium methoxide

Three drops of sodium methoxide were added to a solution of compound I in methanol (2ml) and the UV spectrum was immediately recorded.

2.2.6.2- The UV spectrum in presence of $AlCl_3$

Six drops of aluminum chloride were added to a solution of compound I in methanol (2ml) and the UV spectrum was recorded immediately.

2.2.6.3- The UV spectrum in presence of $AlCl_3$/HCl

Three drops of stock solution of hydrochloric acid were added to the solution in (2.2.6.2) and the UV spectrum was recorded immediately.

2.2.6.4- The UV spectrum in presence of sodium acetate

Excess coarsely powdered anhydrous sodium acetate was added with shaking to acuvette containing (2ml) of the solution of compound I in methanol and the UV spectrum was recorded after two minutes .

2.2.6.5- The UV spectrum in presence of boric acid /sodium acetate

Sufficient powdered anhydrous H_3BO_3 was added with shaking to a cuvette containing the solution in (2.2.6.4) to give a saturated solution .The UV spectrum was recorded after two minutes.

Chapter Three

Results and Discussion

3-Results and Discussion

3.1-Phytochemical screening

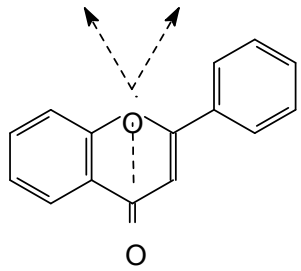
Phytochemical screening of the alcoholic extract of the roots of *Leptadenia heterophylla* revealed the presence of tannins, saponin, terpenes, flavonoids and steroids. Alkaloid and glycosides were not detected. The crude alcoholic extract was fractionated by paper chromatography where compound I was isolated.

3.2-Identification of compound I

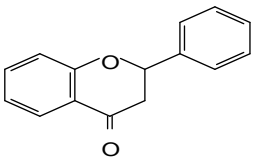
The IR spectrum of compound I(Fig.1) showed ν(KBr) 613,667,779,864(CH,Ar.bending),1035(CO),1417,1460(C=C, Ar.), 1620(C=O), 2856,2925(C-H,aliph.) and 3386 cm^{-1} (OH).

Compound I absorbs in the UV (Fig.2) at λ_{max}(MeOH)265nm (Band II). Such absorption- which originates from a benzoyl chromophore is revealed by flavanones, isoflavones, dihydrchalcones and dihydroflavonols. These classes lack conjugation between the carbonyl function and the B ring, hence

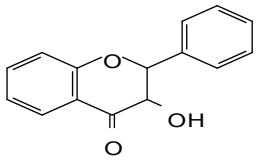
only band II is manivested. Other classes of flavonoids (flavones , flavonols, chalcones and aurones) reveal both band I and II in their UV spectra due to conjugation between ring B and the carbonyl function at C-4 i.e. they possess two chromophores: the benzoyl and cinnamoyl systems.



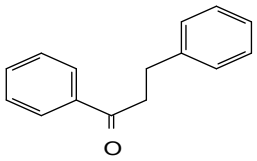
Benzoyl system cinnamoyl system



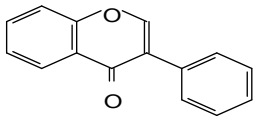
Flavanone



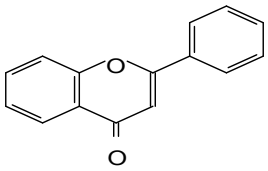
Dihydroflavonol



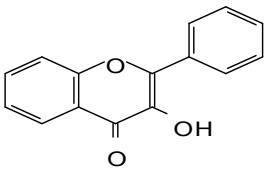
Dihydrochalcone



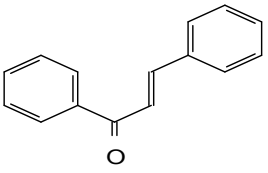
Isoflavone



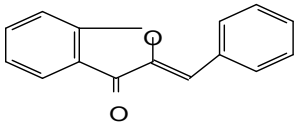
Flavone



Flavonol



Chalcone



Aurone

The appearance of only one band in the UV spectrum suggests that this flavonoid could be: flavanone, isoflavone, dihydrchalcone or dihydroflavonols. But isoflavones give a shoulder in the range 300-340 nm. Such shoulder was not observed in the UV spectrum of compound I. Dihydroflavonols are characterized by a 3-OH function which could be detected by the shift reagent: sodium methoxide.

The shift reagent sodium methoxide is diagnostic of 3- and 4`-OH functions, in both cases it affords bathochromic shifts, but with decrease intensity in case of a 3-OH group.

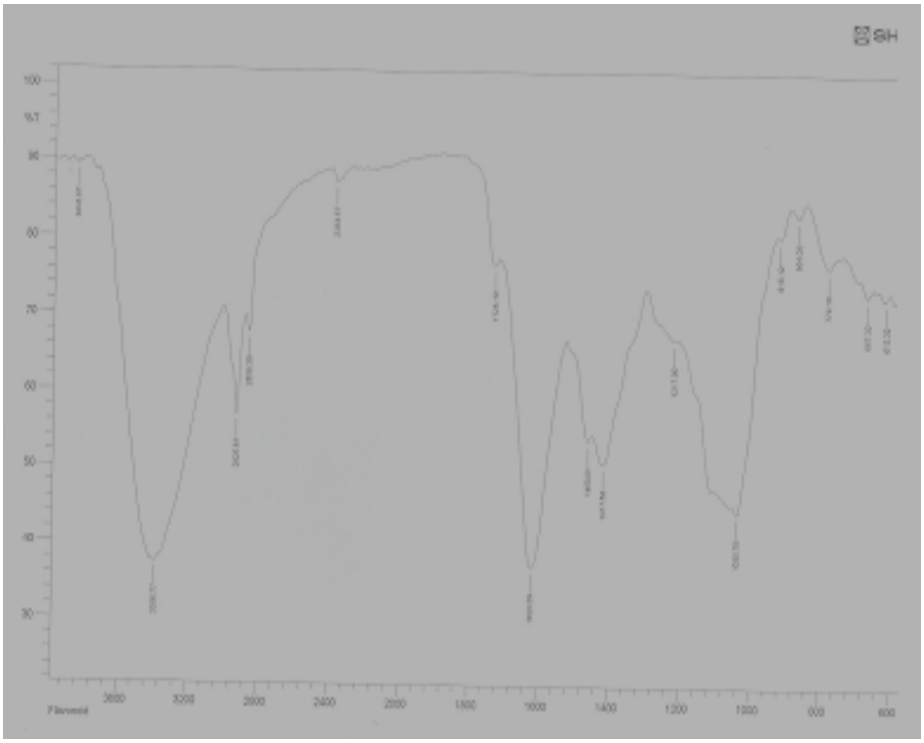


Fig.1:The IR spectrum of compound I

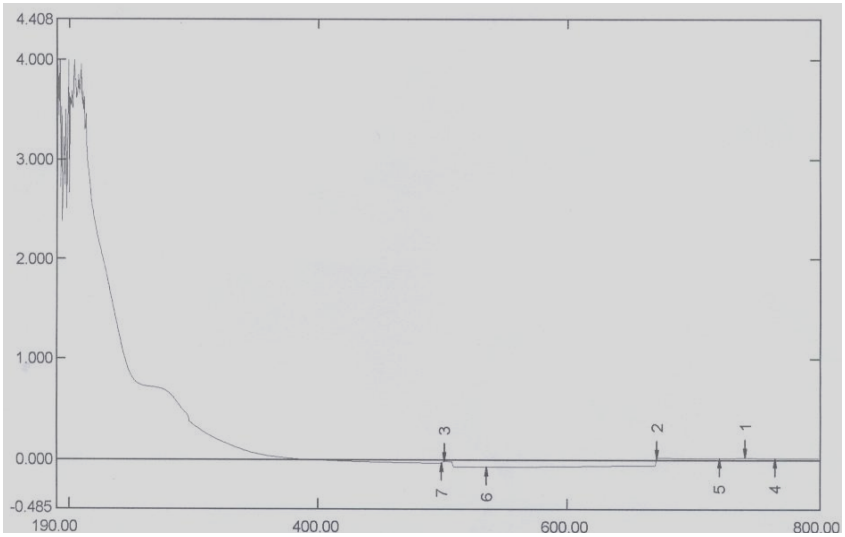


Fig.2: UV spectrum of compound I

The sodium methoxide spectrum of compound I (Fig.3) did not reveal any a bathochromic shift indicating absence of dihydroflavonols. Thus comound I is either a flavanone or a dihydrochalcone. Such classes are distinguishable by their ^{1}HNMR spectra where flavanones afford a double quartet

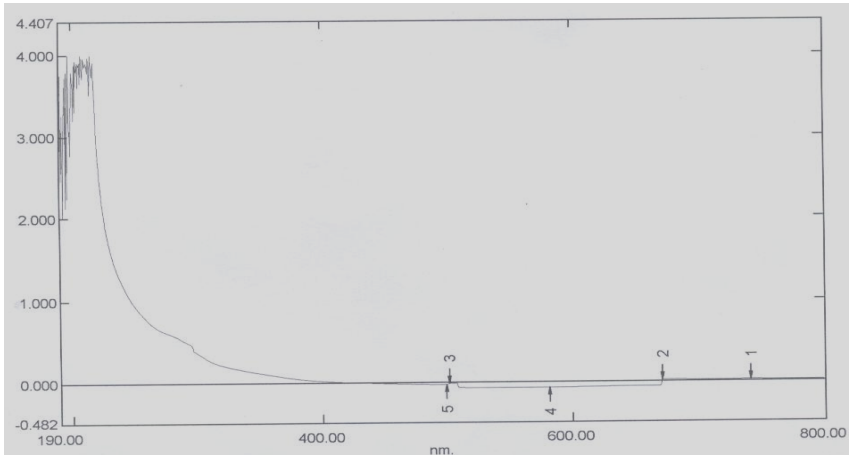


Fig.3:Sodium methoxide spectrum of compound I

(usually merging into a multiplet) around 5.2ppm and aquartet around 2.8ppm. The magneticanlly uneqivalent prottons at C_3 split each other into a double doublet which is further split by C_2 - proton into a double quartet.Also the protons at C_2 split the signal of C_2 proton into a double doublet.

Next the hydroxylation pattern of compound I was investigated via the shift reagents: Sodium acetate and aluminium chloride.

The shift reagent sodium acetate is a weaker base than NaOMe, and ionizes only the more acidic hydroxyl groups. It is useful diagnostic reagent for specific detection of 7-hydroxyl group[1]. When a methanolic solution of compound I was treated with sodium acetate no bathochromic shift was observed (Fig.4). This indicates absence of a 7-OH function.

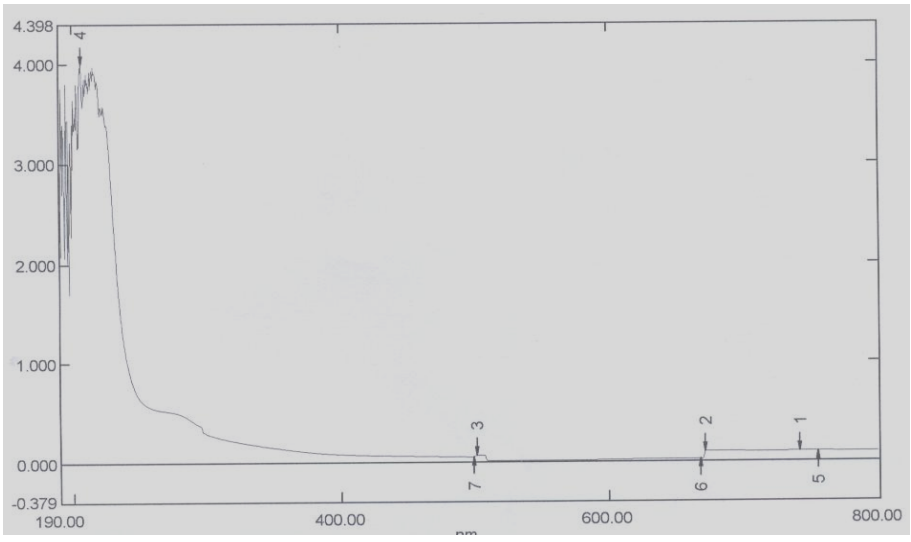


Fig.4: The sodium acetate spectrum of compound I

The shift reagent aluminium chloride forms acid-stable chelates with 3-OH and 4- keto function as well as 5-OH and 4- keto function .It also forms chelates with catechol systems in ring A or B. However, the ortho- dihydroxy system, unlike the 3-OH and 5-OH complexes afford acid-labile complexes[2]. The aluminium chloride spectrum of compound I (Fig.5) gave a 95 nm bathochromic shift. This indicates a 5-OH function since the spectrum was acid –stable (Fig.6).

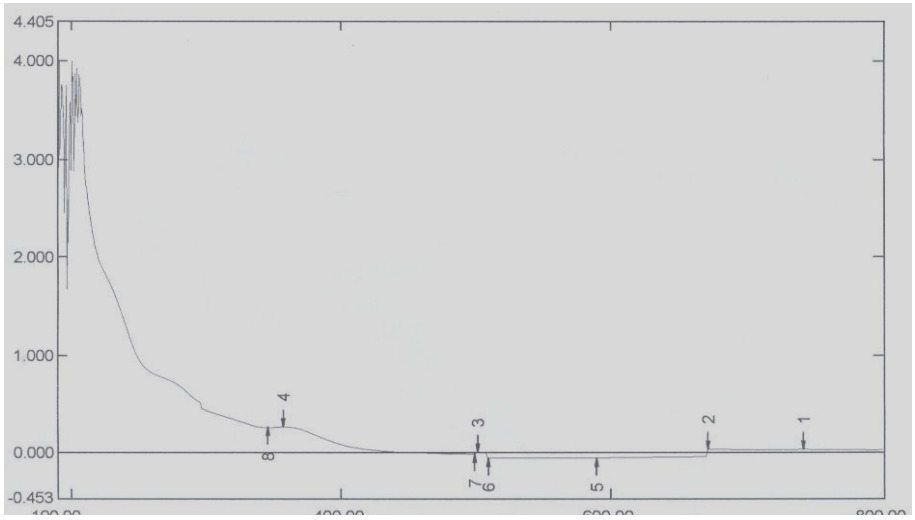


Fig. 5: Aluminium chloride spectrum of compound I

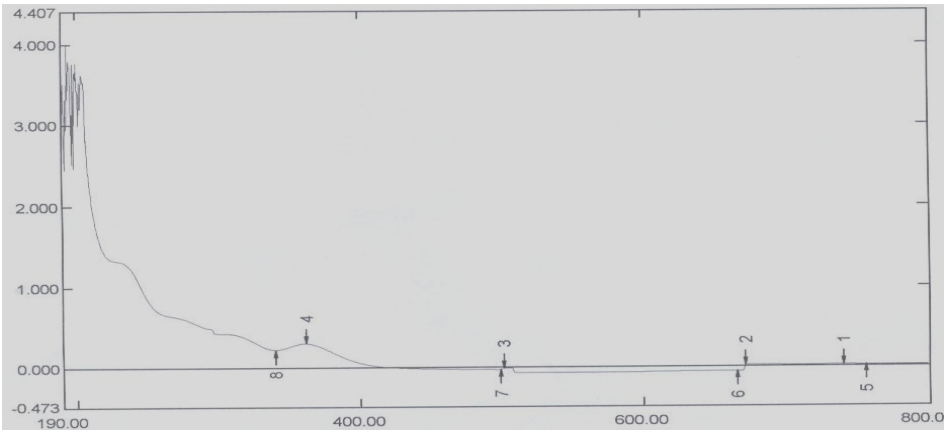
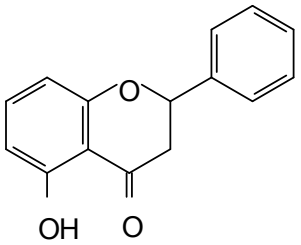
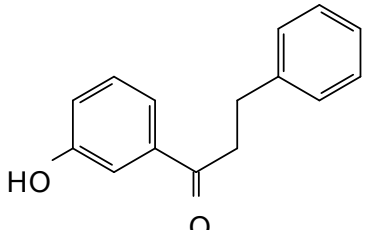


Fig.6: Aluminium chloride/ HCl spectrum of compound I

On the basis of the above cumulative data, compound I is either: a 5-hydroxyflavanone or a 5`-hydroxydihydrchalcone.



5-hydroxyflavanone



5`-hydroxydihydrochalcone

Conclusion

Using solvent extraction and paper chromatography a flavonoid was isolated from roots of *Leptadenia heterophylla*. Spectral studies indicated that the isolate is either a flavanone or a dihydrochalcone. Furthermore, spectral studies with shift reagents indicated a 5-hydroxy function.

Recommendations

The structure of the isolated component may be fully elucidated via more spectral data involving ^{1}HNMR, ^{13}CNMR , MS and 2-dimensional NMR techniques(HMBC,HSQC and ^{1}H-^{1}H COSY NMR).

The flavonoid may be evaluated for antimicrobial, antileshmenial, antimalarial activity.

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