بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

UV Study on the Major Flavonoidfrom *Zingiber officinale* (Ginger)

دراسة طيف الاشعه فوق البنفسجيه للفلافونيد الرئيسي جذور الزنجبيل

A Thesis Submitted in Partial Fulfillment of the Requirements of the Master Degree in Chemistry

By:

Jehad Mohamed Salih Mahjoub

(B.Sc. (honour) chemistry)

Supervisor: Prof.Mohamed Abdel Karim Mohamed

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Sudan University of Science & Technology

College of Graduate Studies

بسم الله الرحمن الرحيم

جامعة السودان للعلوم والتكنولوجيا

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في الزنجبيل.....

وهي منتج فكري أصيل . وباختياري أعطى حقوق طبع ونشر هذا العمل لكلية الدراسات العليا - جامعه السودان للعلوم والتكنولوجيا، عليه يحق للجامعه نشر هذا العمل للأغراض العلمية .

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الآية

قال تعالي:

اقْرَأْ باسْمِ رَبِّكَ الَّذِي خَلَق{١}خَلَقَ الْاِنسَانَ مِنْ عَلَقٍ {٢} اقْرَأْ وَرَبُّكَ الْأَكْرَمُ {٣}الَّذِي عِلَّمَ بالْقَلَمِ{٤} عَلَّمَ الْاِنسَانَ مَا لَمْ يَعْلَمْ{٥}

صدق الله العظيم

سورة العلق الايات من ١- ٥

Dedication

To

my parents

and brothers

Acknowledgement

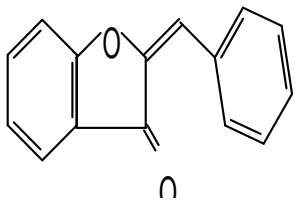
I thank Almighty Allah for giving me the courage and the determination, as well as guidance in conducting this research study, despite all difficulties.

I wish to extend my utmost gratitude to my supervisor, Professor Mohamed Abdel Karim Mohamed for his tremendous help, advice and support during the period of this study.

I would like to thank the laboratory staffs Sudan university of sciences and technology for their kind help and infinite support. Finally, I thank all those who assisted, encouraged and supported me during this research.

Abstract

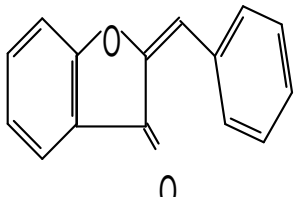
In this study the extract of *Zingiber Officinale* (ginger) was subjected to phytochemical screening which revealed the presence of glycosides, steroids and flavonoids. The crude extract was purified by column chromatography followed by thin layer chromatography .The characterization of isolate was accomplished by UV study; the UV data suggest a pattern characteristic of aurones. The following tentative structure was proposed:



خلاصة البحث

في هذه الدراسة اخضع مستخلص جذور نبات الزنجبيل لمسح فيتوكيميائي اوضح وجود: الجلايكوسيدات،الاستيرودات والفلافونويدات. وقد تمت تنقية المستخلص الخام بكروماتوغرافيا العمود و كروماتوغرافيا الطبقة الرقيقة .

وتم تحديد التركيب المبدئي عن طريق الأشعة فوق البنفسجية وأظهرت هذه المطيافيه ان هذا الفلافونويد ينتمي لطائفة الاورونات وقد اقترح التركيب المبدئي التالي:



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1. Introduction

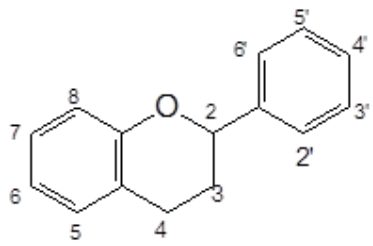
1.1. General approach

Plants have been associated with the human health from time immemorial and they are the important source of medicines since human civilization [1]. Plants still remain one of the major sources of drugs in modern as well as in traditional systems of medicine. Plant secondary metabolites are important sources of many food ingredients and phytochemicals [2]. Plants produce several secondary metabolite compounds including: alkaloids, cyanogenic glycosides, glucosinolates, flavanoids, saponins, steroids and terpenoids to protect themselves from the continuous attack of naturally occurring pathogens, insect pests and environmental stresses[3].

Flavonoids are an extensive group of polyphenolic compounds that occur commonly in plants. They are prominent secondary plant metabolites that are present in dietary components, including fruits, vegetables, olive oil, tea and wine. As a group, flavonoids contain more than 8000 known compounds and this number is constantly growing due to the great structural diversity arising from various hydroxylation, methoxylation, glycosylation and acylation patterns. Many flavonoids are known to show biological activities such as antiinflammatory, antiallergic, antithrombotic, antibacterial, antifungal and

antitumoral properties[4] .They are also active as anti-oxidants although the *in vivo* anti-oxidant activity is very limited due to weak absorption (around 5%) in the small intestine, together with rapid metabolizing and excretion [5].

The term "flavonoid" is generally used to describe a broad collection of natural products that include a C_6-C_3-C_6 carbon framework, or more specifically phenylbenzopyran functionality. 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: the flavonoids (2 phenylbenzopyrans) (1), isoflavonoids (3-benzopyrans) (2), and the neoflavonoids (4-benzopyrans) (3). These groups usually share a common chalcone precursor, and therefore are biogenetically and structurally related.



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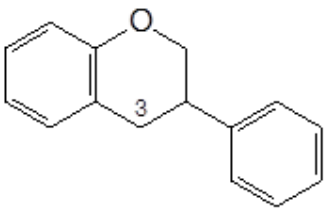

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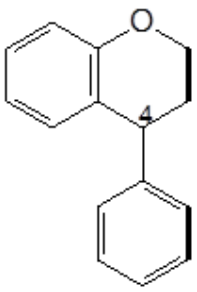
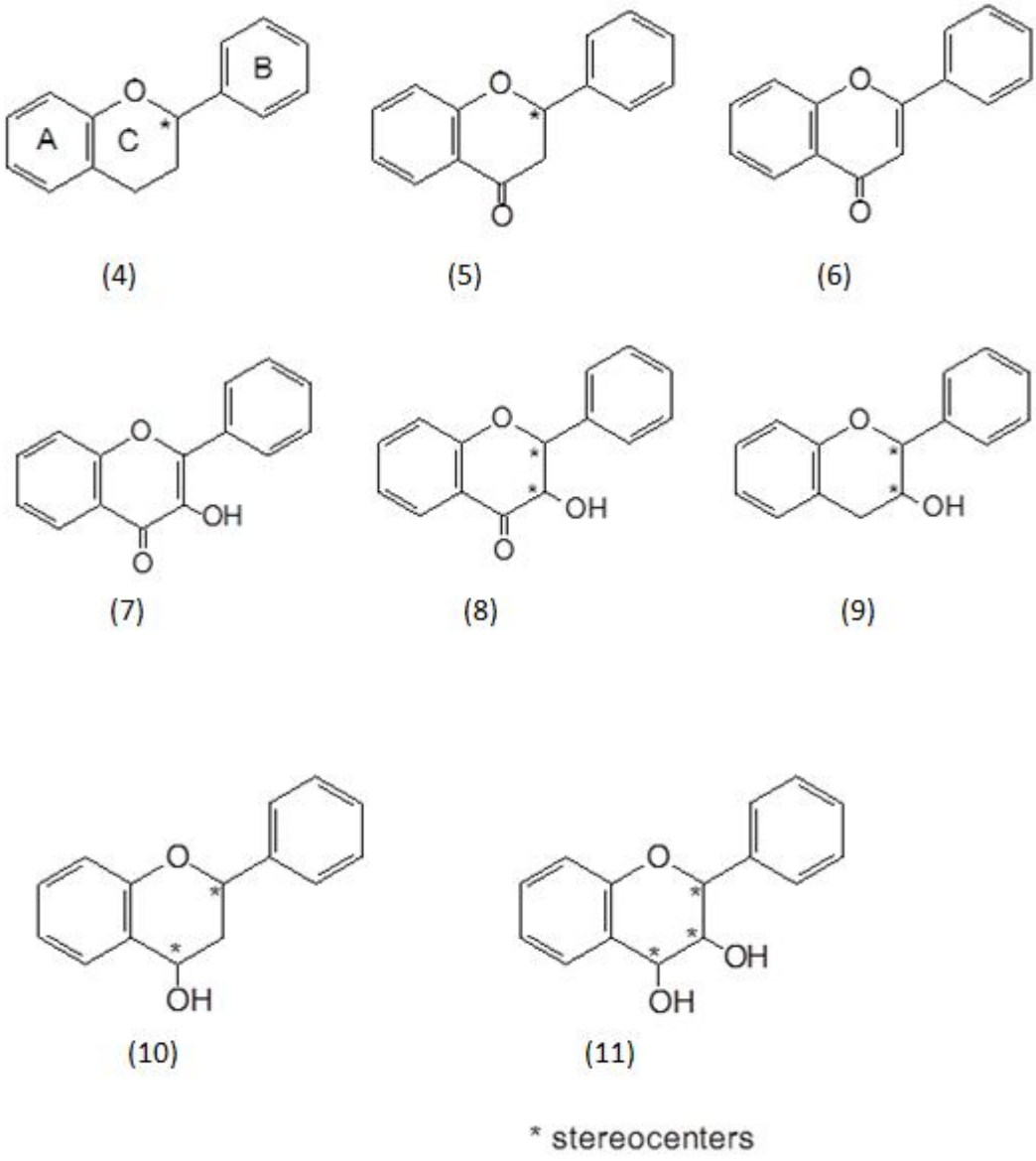


(3)

1.2. 2-Phenylbenzopyrans(C_6-C_3-C_6 backbone)

Based on the degree of oxidation and saturation present in the heterocyclic C-ring, the flavonoids may be divided into the

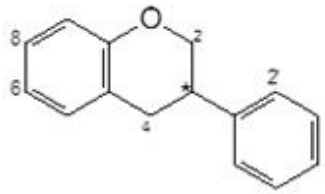
following groups: flavans(4) , flavanones(5) , flavones(6), flavonols(7), dihydroflavonols (8), flavan-3-ols(9), flavan-4-ols(10), flavan-3,4-diols (11).



1.3. Isoflavonoids

Isoflavonoids are subdivided into the following groups: isoflavans (12),isoflavone (13) ,isoflavanones(14),isoflav-3-enes(15),isoflavanols(16) ,rotenoids (17), comuestanes(18), 3-

arylcoumarins (19) ,coumaronochromenes(20), coumaronochromones(21) , ptrocarpans(22).



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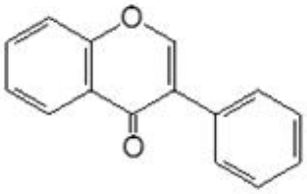

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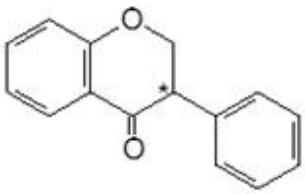

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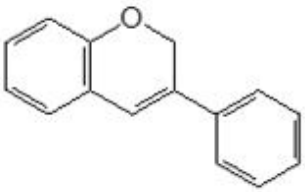

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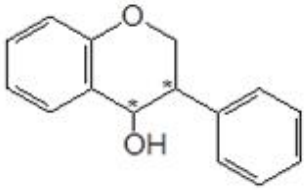

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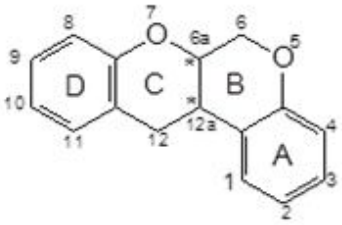

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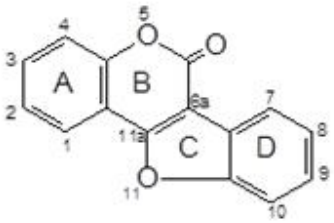

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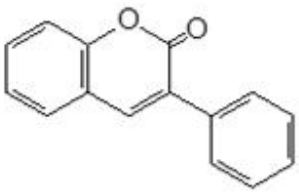

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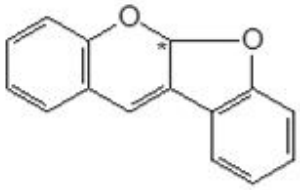

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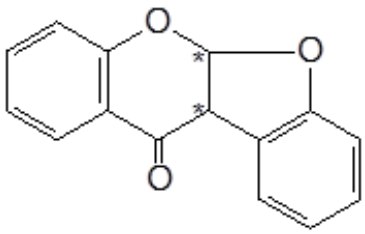

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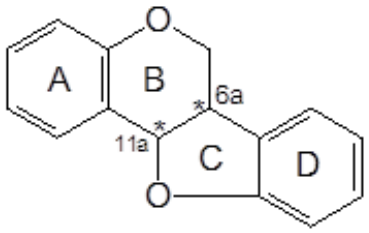


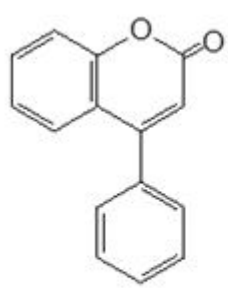
(22)

*stereocenters

These compounds constitute a distinctive subclass of the flavonoids. They possess a 3-phenylchroman skeleton that is biogenetically derived by 1, 2-aryl migration 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[5].

1.4. Neoflavonoids

Neoflavonoids comprise: the 4-arylcoumarins (4-aryl-2*H*-1-benzopyran-2-ones) (23), 3, 4-dihydro-4-arylcoumarins (24), and neoflavenes (25).They are structurally and biogenetically closely related to the flavonoids and the isoflavonoids[6].



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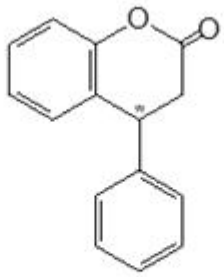

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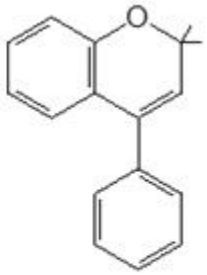
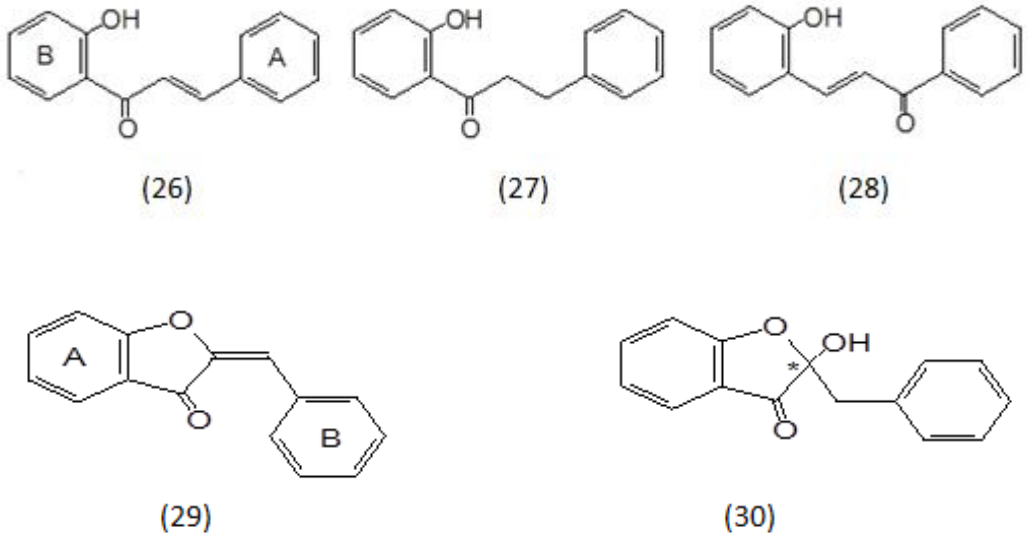


(25)

*stereocenters

1.5. Minor Flavonoids

The term minor flavonoids is used to describe natural products such as chalcones and aurones which also contain a C_6-C_3-C_6 backbone. These compounds include the 2′-hydroxychalcones (26), 2′-OH-dihydrochalcones (27), 2′-OH-*retro*-chalcone (28), aurones (2- benzylidenecoumaranone) (29) and auronols (30)[6].



(26) (27) (28)

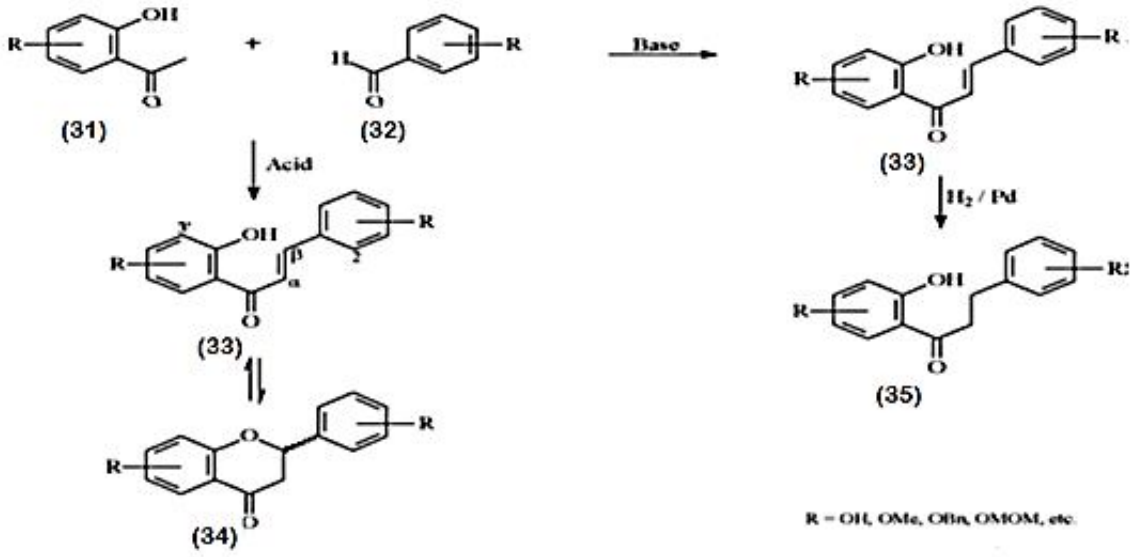
(29) (30)

*stereocenters

1.6. Chalcones, Dihydrochalcones, and Racemic Flavonoids

Chalcones and dihydrochalcones are considered to be the primary C_6-C_3-C_6 precursors and constitute important intermediates in the synthesis of flavonoids. Chalcones are readily accessible via two well-established routes comprising a base-catalyzed aldol condensation or acid-mediated aldolization of 2-hydroxyacetophenones(31)and benzaldehydes (32)(Scheme

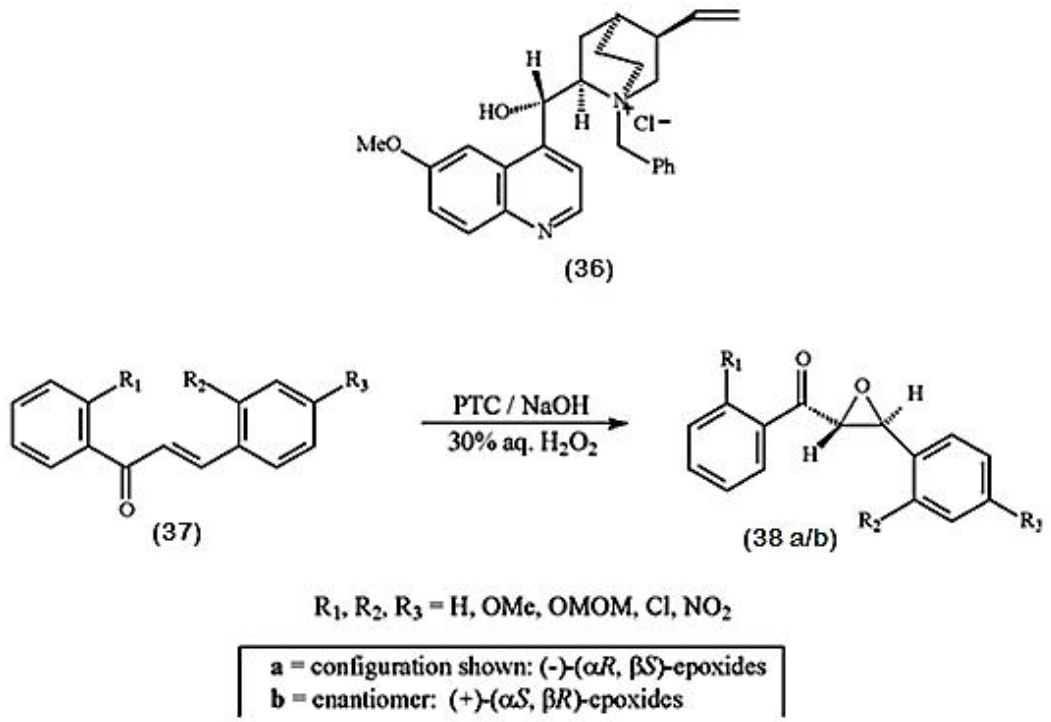
1.1)[6]. The base-catalyzed aldol condensation is usually the preferred route toward chalcone (33) formation, since under acidic conditions cyclization of the ensuing chalcone leads to formation of corresponding racemic flavanones (34) [7]. Dihydrochalcones (35)are generally obtained via reduction (H2/Pd) of the preceding chalcones (Scheme 1.1).



Scheme 1.1: Acid- and base-catalyzed synthesis of chalcones, racemic flavanones, and dihydrochalcones.

Asymmetric epoxidation of olefinic bonds plays a crucial role in introducing chirality in the synthesis of several classes of optically active natural compounds. Sharpless and Jacobson [8, 9]developed viable protocols for the enantioselective epoxidation of allylic alcohols and unfunctionalized olefins. However, attempts regarding the enantioselective epoxidation of α, β-unsaturated ketones, in particular chalcones, have met with

limited success. Wynberg and Greijdanus [10] first reported the utilization of quinidine benzylchloride (36) as chiral phase transfer catalysts (PTC). Since then, the use of PTC has emerged as one of the preferred methods for the asymmetric epoxidation of α, β-unsaturated ketones and led to the first stereoselective synthesis of (-) - and (+)-*Trans*-chalcone epoxides (38a/b)[yield: 38–92%; enantiomeric excess (ee): 25–48%] [10].



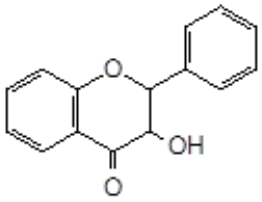
Scheme 1.2: Epoxidation of chalcones

This resulted in several investigations of alternative catalysts and reaction conditions to enhance the enantioselectivity of the epoxidation of enones. However, these attempts were limited to nonchalcone enones and a few non- and monooxygenated

chalcone substrates, which lacked natural product oxygenation patterns[11].

1.7. Dihydroflavonols

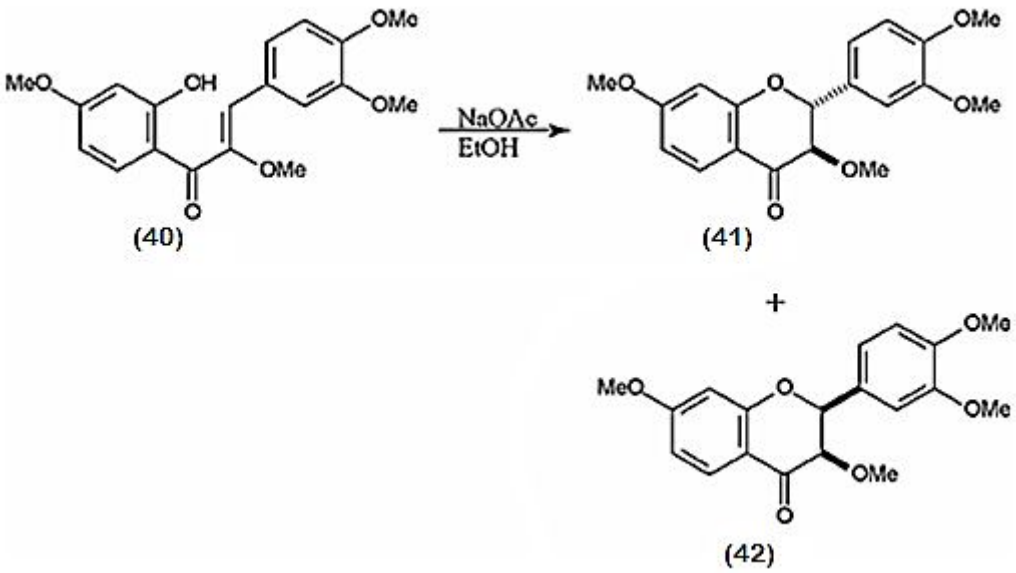
Dihydroflavonols (39) are simply flavanones bearing a 3-OH function.



(39)

Although the Algar-Flynn-Oyamada (AFO) protocol and the Weeler reaction were mainly used for the synthesis of aurones,[12] it was demonstrated that these reactions can be adapted for the formation of racemic dihydroflavonols in moderate to good yields.[13, 14]

Cyclization of 2′-hydroxy-α,3,4,4′-tetramethoxychalcone(40)with sodium acetate in ethanol furnished both 3,3′,4′,7-*O*-tetramethyl-2,3-*trans*-(41)and 3,3',4',7-*O*tetramethyl- 2,3-*cis*-dihydroflavonols (42)in 22% and 11% yields, respectively . However, this method was not applicable to cyclization of α-OH-chalcones[15].



Scheme 1.3 Chalcone cyclization with NaOAc in EtOH to yield trans- and cis-dihydroflavonols.

1.8. Flavones

The parent unsubstituted flavone, produced apparently by a biosynthetic pathway, occurs in the farina on species of *Primula* and the closely related *Dionysia* [16]. 2' -Hydroxyflavone and 5, 2'–dihydroxyflavone have been detected in the secretion of the glandular cells of *Primula florindae* flowers[17].

Apigenin and luteolin, free and as glycosides, are the most widely occurring flavones. The A-ring of the great majority of flavones is derived from phloroglucinol and the B-ring is oxygenated in the 4'or3', 4'- or 3', 4', 5' -positions as expected from their established acetate-shikimate biosynthetic origin.

In a survey[18] of twelve highly specialized herbaceous families, it was found that 6-hydroxyluteolin is present in the majority as a leaf constituent, accompanied occasionally by its 6-methyl and 6,4'-dimethyl ethers; luteolin was frequently present, together

occasionally with scutellarein and pectolinarigenin.The ability of angiosperms to hydroxylate flavones in the 6-position apparently arose relatively late in evolutionary time[18] . Of special biogenetic interest is the occurrence in the seeds of *Casimiroa edulis* of five flavones with the common feature of 5,6-dimethoxy or 5-hydroxy-6-methoxy groups; two of them contain methoxyl groups in the 3' and 3',5'-positions, representing a B-ring substitution difficult to explain by a shikimic acid origin.

Some studies invalidated some of the earlier structures proposed for some flavones; zapotin and zapotinin are two examples. The structure (5,4'-dihydroxy-6, 7 ,8,3'-tetramethoxyflavone), ascribed to a pigment isolated from *Citrus reticulata*, has been shown to be erroneous by comparison with the synthetic compound [19].It is probable that this citrus flavone is identical with gardenin D(5,3'-dihydroxy-6,7,8,4'-tetramethoxyflavone). NMR and MS data have shown that gardenin is a 5-hydroxy-6, 7, 8, 3', 4', 5'-hexamethoxyflavone [19].

Flavone bisulphate salts are new class of flavone pigments, detected in 17 of 31 palm species, which may be of value as taxonomie markers. In *Mascarena verschaffeltii* one of the isolated flavones is the 7-K bisulphate salt of luteolin 3'-glucoside [20].

Only four C-methylflavones have been found as natural products.Strobochrysin (6-methylchrysin) occurs in the heartwood of *Pinusstrobus* and other pine species[21].Although both 6- and 8-methyl-5, 7-dihydroxyflavanones (cryptostrobin and stropopinin) occur in the fern *Matteucia orientalis,* but no 6, 8-dimethylchrysin was encountered. Likewise, 6, 8-dimethyl-5, 7-dihydroxyflavanone (desmethoxymatteucinol) and the 4'-methoxy derivative (matteucinol) occur in Matteucia *orientalis,* but 6, 8-dimethylchrysin and 6, 8-dimethylacacetin are unknown as natural products. 5-Hydroxy-7,4'-dimethoxy-6-methylflavone and the 6,8-dimethyl derivative(eucalyptin) have been isolated from the heartwood of *Eucalyptus torelliana* (Myrtaceae), and 5,4'-dihydroxy-7-methoxy-6,8-dimethylflavone(sideroxylin) from *E. sideroxylon* .Eucalyptin was first identified in the waxes of six species of *Eucalyptus* [22]. Eucalyptin has been synthesized by the action of methyl iodide on 8-methylapigenin 4'-monomethyl ether in methanolic KOH [23]. A route to 8-methylchrysin is the catalytic reduction of Mannich bases from 7-hydroxy-5-methoxyflavone and formaldehyde; acid treatment results in demethylation and partial rearrangement to strobochrysin [24].

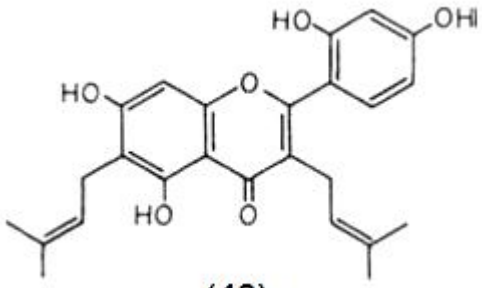
Among the 400 or more phenolic compounds with isoprenoid suhstituents so far isolated from plants, only one is a flavone (as distinct from flavonol), if the flavones of *Artocarpus* and *Morus*s pecies are excluded. The solitary example is 8-prenyl-

luteolin occurring in *Xanthium spinosum* and *X. strumarium* (Compositae) [25]. Excluding the *Artocarpus* and *Morus* flavones again, flavonoids of this type are largely restricted to two families: Leguminosae and Rutaceae.

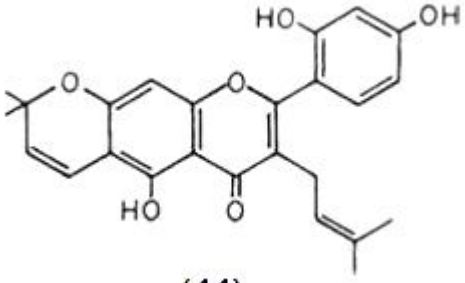
Progressive solvent extraction and chromatography of extracts of *Artoearpus heterophyllus* have shown that this wood is a rich source of flavones with isoprenoid substituents, all of them with the common feature of hydroxyl groups (free or protected) in the 5, 7, 2', 4'-positions[26]. In addition to a new flavanone, artocarpanone (5, 2', 4'-trihydroxy-7-methoxyflavanone), eight new flavones have been isolated, and six of them carryisoprenoid substituents as C-prenyl or modified groups.

Morus Alba bark contains four flavones closely related to the *Artocarpus* pigments: mulberrin (43), mulberrochromene (44), cyclo- mu1berrin (45) and cyclomulberrochromene (46) [27]. The relation of the *Morus* bark flavones to each other and toartocarpin and cycloartocarpin has been shown by interconversions. Mulberrin is apparently the biosynthetic

Precursor of the other *Morus* barks pigments as well as artocarpin and cycloartocarpin[28].



(43)



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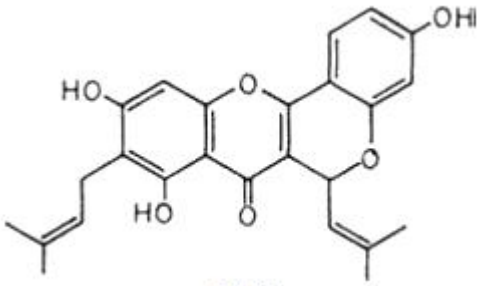

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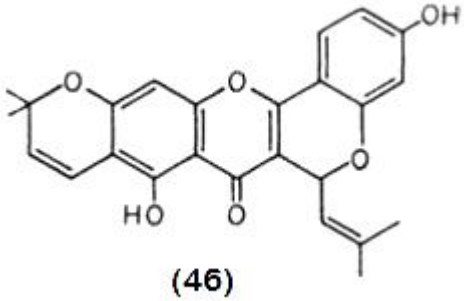
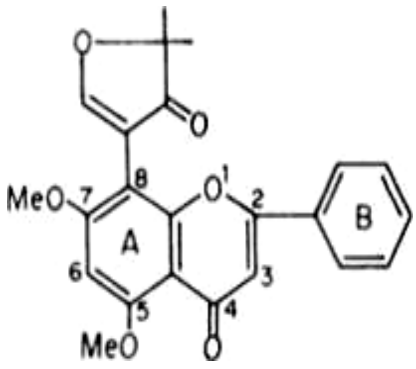


(46)

Tachrosin (47) and stachyoidin (48), isolated from *Tephrosia polystachyoides* (Leguminosae), can be biogenetically derived from 8-prenylchrysin by oxygenation steps and the introduction of one-carbon and two-carbon units[29].



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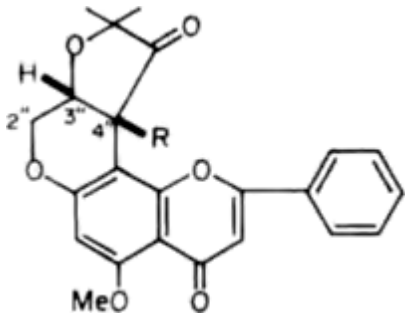
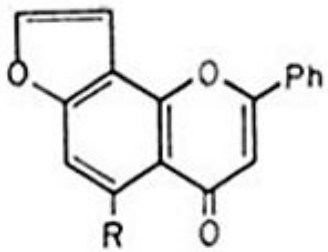


(48)

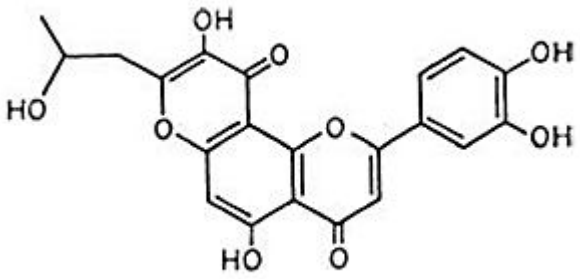
The parent furanoflavone (48; R = H), lanceolatin B, occurs in the roots and barks of *Tephrosia lanceolata.* Karanjin. Pinnatin (49; R = OMe) and gamatin (3', 4'-methylenedioxypinnatin), together with karanjin and pongapin (3', 4' methylenedioxykaranjin), have been isolated from the roots of *Pongamia pinnata;* their structures have been confirmed by synthesis[30].



(49)

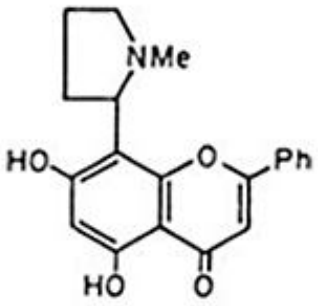
Arthraxin (50) occurs together with luteolin and its 7-glucoside in the leaves and sterns of the herb *Arthraxon hispidus* Makino (Loranthaceae). All three have also been isolated from *Miscanthus tinctorius* Hackel (Compositae). It is clear that arthraxin is derived from luteolin by the attachment of three

acetate units and subsequent reduction, hydroxylation and cyclization[31].



(50)

The alkaloidal flavones- ficine (51) was isolated and identified. The MS of ficine did not show a molecular ion, but was equivalent to the sum of the spectra of chrysin and N-methylpyrrole. All the expected signals for (51) were observed in the NMR spectrum[32].



(51)

Flavones undergo a photoreaction with diphenylacetylene. Under appropriate conditions the Udenfriend reagent (ascorbic acid and ferrous ion chelate) hydroxylates flavones by a radical mechanism to 3; 4; -dihydroxyflavone and by an ionic process

to flavonol. The reaction of flavone with substituted hydrazines has been reinvestigated [33]. When the carbonyl reagent is not too basic to prevent the use of acid catalysts, the normal hydrazone is formed, but with increasing basicity the products are pyrazoles.

Both flavones and flavonols can be synthesized by the Allan-Robinson reaction and the Baker Venkataraman rearrangement; a useful modification is the treatment of an o-hydroxyacetophenone with an aroyl chloride and anhydrous K_2CO_3 in boiling acetone, when the dibenzoylmethane (or the3-substituted flavone in the case of a γ-substituted acetophenone) is directly formed in good yield. For flavones, as distinct from flavonols, the selenium dioxide oxidation of 2'-hydroxycha1cones is most widely used; one advantage is the absence of 3-aroylation, a complicating factor in the Allan-Robinson and Baker-Venkataraman reactions. When the monobenzoate of 2, 6-dihydroxy-4-methoxy-acetophenone is heated at 500^{o}C with KOR in pyridine, the product is the 2-hydroxyflavanone[34].

Gabor[35] Has reviewed trends in research on the pharmacodynamic effects of the flavonoids, mostly rutin and its derivatives. Flavone itself possesses coronary dilating action. The condensation product of 7-hydroxyflavone and ethyl bromoacetate has been used clinically in Italy as a coronary vasodilator; it is stated to be non-toxic and more active than

nitroglycerine or khellin[36]. There appears to be continued interest in flavones with a 7-OCH_2 COOR group as coronary vasodilators. The ethyl ester and the hydrazide have been claimed as contraceptive drugs. The 1, 3-dimethylxanthine-7-acetic acid salt or 7-ß-dimethylaminoethoxyflavone ('Perflavon') is reported to have antiphlogistic, choleretic, spasmolytic and antihistamine activity. 7-Hydroxy-8-dialkylaminomethylflavones, prepared by a Mannich reaction on 7-hydroxyflavone, are heart stimulants. The Mannich reaction on o-hydroxydibenzoylmethane yields a 3-aminomethylflavone, and such compounds (e.g. 3-dimethylaminomethylflavone) have anticonvulsant, analgesic and bronchodilator activity .Baiealein phosphates are useful for treatment of allergie diseases. Acacetin, administered orally to mice at 25-100 mg kg1, decreases formalin-induced inflammation and reduces capillary fragility. Administered intravenously to rabbits at 25 mg kg^{-1}, it increases urinary excretion by 75% in the first 25-30 min. The preparation of luteolin 3', 4'-dimethyl ether by the Baker-Venkataraman reaction and its demethylation to luteolin are described in a German patent, which refers to their non-toxic character and their hypocholeretic and spasmolytic properties[37].

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 [38].This practice is justified in that much of their chemistry, analysis, synthesis, and reactions have

a common theoretical basis. However, today it is apparent that this simple difference in structure is of considerable biosynthetic, physiological, phylogenetic, chemosystematics, pharmacological and analytical significance. Preliminary information on flavonols present in a plant extract can be obtained by two-dimensional paper chromatography. About 30 such chromatograms yield sufficient quantities, either of a pure flavonoid for UV spectral analysis, or of a partially purified product for one-dimensional paper chromatographic separation. Lists of R_F values are available for different developing systems. Column chromatography, though of inferior separating efficiency, is the method of choice whenlarger quantities of flavonoids are required[39].Of the various TLC procedures that can be used, that on silica impregnated with lead acetate is of interest since it is based on the earlier isolation procedure for flavonols by precipitation as their lead salts. GLC of flavonol methyl ethers on low loaded columns is feasible, no sign of decomposition having been detected. The same is true for trimethylsilyl ethers of flavonol, which have much higher retention times than those of the related flavones. Retention times are related to number of hydroxyls in ring B. GLC is particularly useful when coupled with MS analysis. Finally, paper electrophoresis in borate buffer not only rapidly separates flavonols from their glycosides, but relative mobilities may even reveal some structural features; it must be remembered,

however, that flavonols are oxidized very quickly at alkaline pHs[39]. Should any of the above methods of isolation or separation, however, be either too laborious or ineffective, fractional sublimation *in vacuo* may prove useful [40]. Colour reactions are important during the isolation of flavonols and they will even distinguish between gossypetin and quercetagetin, two isomers which are usually difficult to separate chromatographically. Given sufficient quantities of compound, the tradition of colour tests can be applied and may contribute significantly towards knowledge of structural details[41]. The reaction of flavonols with diazonium compounds gives, as by-products, hydroxyphenylazo derivatives through displacement of the 2-phenyl group, and this is therefore not recommended as a spot test.[42]

Flavonols display a range of colours when chromatographed on paper and viewed in UV light (see Table 1.1). Flavonols substituted in the 3-position appear as dark spots or have a dark brown fluorescence , while derivatives which do not have a free hydroxyl at C-5 are generally distinguished by intense fluorescent colours (Table 1.1). This fluorescence has sometimes erroneously been considered to be characteristic of 5-deoxyflavonols in general[43]. Additional substitution may however alter the situation; e.g.,6-hydroxylation, but not 6-methoxylation, apparently quenches the fluorescence of a flavonol , and quercetagetin as weIl as gossypetin appear dull

black in UV light with the Colour unchanged by ammonia. Confirmation is thus indispensable and may be sought by two traditional techniques. Addition of acetic anhydride to the flavonol sampIe quenches the fluorescence of 5-hydroxy derivatives , while addition of citric acid to zirconium complexes of the flavonol destroys the yellow colour of the 3-alkoxy-5-hydroxy derivative and preserves the yellow colour of the 3-hydroxyderivative . Ozone-induced chemiluminescence approximates in sensitivity to the fluorescence methods and can be used to detect as little as 3 mg of quercetin [44].

Table 1.1 Relationship between color on chromatograms and flavonol substitution *

UV	UVINH$_3$	3	5	4'
yellow	Yellow	OH	OH	
fluorescent	fluorescent	OH	H/OMe	
yellow	Yellow			
fluorescent	fluorescent	OMe	H/OMe	
light blue	yellow-green			
deep purple	yellow-green	OMe	OH	OH
deep purple	deep purple	OMe	OH	H/OMe

Finally, the flavonol-containing spots can be eluted direct1y from such chromatograms and UV spectral analysis carried out[45].

The quantitative determination of flavonols usually relies on spectrophotometric or spectrofluorometrie methods. These may be applied in solution or on chromatographic spots, either

directly or to the tin and aluminium chelates. Fundamental studies on the stoicheiometry of such complexes have been carried out. The sensitivity of these methods is such that they may be used to analyse air-borne bacterial (phosphatase) activity through quantitative determination of 3-hydroxyflavone enzymically liberated from its phosphate. Less commonly used methods of flavonol analysis are based on complexometric, microcoulometric, polarographic and potentiometric techniques[46]. Flavonols are more susceptible to oxidation than other flavonoids so that chemical methods of determination often rely on oxidants. A special one, applied so far only to the microdetermination of kaempferol, is $PtC1_4$ in NaOH which is back titrated after cleaving the compound into formaldehyde, formic and p- hydroxybenzoic acids . Another oxidant, Cu (II) ion in propionic acid, is used in the titration of flavonols in non-aqueous medium[47]. The progress of the oxidation may be followed potentiometrically or spectrophotometrically.

The reducing character of the substrate is determined by the free hydroxyl on the heterocycle and enhanced through hydroxylation at the 5- and 4'-positions. O-Diphenolic functions on ring B are not oxidized, as they would if the 2, 3-bond was saturated. However, an o-diphenolic function at 3', 4' can be separately determined spectrophotometrically by measuring the intensity of the red colour produced by $NaN0_2$ /$NaMo0_4$/AcOH[48].

The reliability of the structural analysis of flavonols, traditionally based on UV spectrophotometry and alkaline cleavage, has been considerably enhanced in the past decade by additional instrumental techniques, especially PMR spectrometry.

The exploitation of UV spectra in the analysis of flavonols has been reviewed by Jurd[49]. Flavonols exhibit two major absorption peaks band me (358 to 385 nm) and band 11 (240 to 280 nm). These bands are associated respectively with the B-ring cinnamoyl system and the A-ring benzoyl system, and only flavones, among other types of flavonoids, give comparable spectra. 3-Hydroxyflavones can be usually recognized, nevertheless, by the position of band I (352 to385 nm). Distinction between 3-O-substituted flavonols and flavones by UV, however, is not possible, since the band I range of both classes overlap in the 328 to 357 nm region.

The exact wavelength of band I of 3-hydroxyflavones (mean value 371. 5 ± 1.1 nm) and of 3-methoxyflavones (mean value: 350 ± 1.9 nm) depends chiefly on the oxygenation of ring B and, to a lesser extent, of ring A; while the wavelength of band II depends on the oxygenation of ring B. Table 1.2 shows that this statement must be qualified. With respect to band I, introduction of each additional hydroxyl into ring B corresponds to regular shifts of about +6 nm, while introduction of additional hydroxyls into ring A produces either hypso- or bathochromic

effects of about 10 nm. The oxidation of ring B additionally influences the shape of band II, 4' –oxygenated derivatives showing only one peak, while 3', 4' (and to a lesser extent3', 4', 5')-oxygenated flavonols usually exhibit a maximum (IIb) with a shoulder (lIa) at the long wavelength side[50].

Exchange of a hydroxyl for a methoxyl in a flavonol produceschanges in the positions of bands I and II which may be diagnostically useful. While the number of known correlations for OH/OMe exchange at positions 6, 8 and 3' is not significant, the remaining data of Table 1.3 may be used with confidence. Especially valuable for identification purposes is the knowledge that etherification of a 5-hydroxyl produces a hypsochromic shift of both bands, while etherification of a 3-hydroxyl produces such a shift only of band I.

Table 1.2 Dependence on the hydroxylation pattern of the wavelength (nm) of UV maxima of 3-hydroxyflavones[50]

Bands	Hydroxylation pattern of rings				
	B		**A**		
	- -	**7**	**5,7**	**5,6,7**	**5,7,8**
	344		360		
I	4'	357	368		380
	3',4' 368		374	365	368
	3',4',5'	368	378		
	- 239		268		
II	4' 258		270		275
	3',4' 250	253	255	259	262
	3',4',5'	250	258		

The location of some hydroxyls, which can thus be obtained by comparing the spectra of the flavonol and of its O-methylethers,

Table 1.3 Effect of O-methylation on the UV spectra of flavonols [51]

OH-OMe at C-	**Shift (nm) of Band I**	**Band II**
3(5-OH)	-12 to -17 o	
3 (5-H/OR)	-21 to -25	small neg
5	-5 to -15	-5 to -15
6	+8	-1
7	+2 to-5	+2 to-3
8	+2 0	
3'	+4 to -6~	-1 to-3
4'	-3 to -10	-1 to-2

Should always be checked by observing UV spectral shifts in presence of inorganic salts. NaOMe, a strong base which ionizes hydroxyls at all positions, is useful for the diagnosis of 4' –OH,3-0H (band I shift of +40 to 65 nm without decrease of intensity), 3-0H-4'-OR (band I shift of +50 to 60 nm with decrease of intensity), 3,4'-diOH(degeneration of spectrum) systems. Alkali sensitivity, however, may also be due to the presence of catechol or pyrogallol groups. NaOAc, a weaker base, ionizes only hydroxyls at positions 3, 7 and 4', and is useful for the diagnosis of 7-hydroxyls (band II shift of +5 to20 nm). A band I shift is, however, also observed, and thus conclusions concerning the presence of a free 4' -hydroxyl by this technique are valid only if both the 7- and the 3-hydroxyls are etherified. If the 7-hydroxyl is part of a 5, 6, 7- or 5, 7, 8-trihydroxysystem, the NaOAc spectrum must be measured immediately after introduction of the salt into the cuvette, because of the alkali sensitivity of the compound. Should this possess 3, 3', 4' –trihydroxy or even 3, 4' -dihydroxy-3' -methoxy-groups, decomposition is very rapid.

H_3BO_3 in the presence of NaOAc will chelate with o-dihydroxyls at all locations of the flavonol nucleus, except at C-5,-6, producing a12 to 30 nm bathochromic shift of band I. The presence of catechol systems can also be ascertained using $AlCl_3$, a reagent which also complexes with 3-hydroxy- and 5-

hydroxy-4-carbonyl groupings.The data for flavonols are summarized in Table 1.4.

Infrared spectral studies, as an aid to structure elucidation even with moderately substituted derivatives, are complicated by super position of effects. The frequency of the C-H out-of-plane bending modes is largely independent of the nature of the substituents and serves for the detection of 3', 4'-, 4'- and unsubstituted B-rings. The frequency and intensity of the O-H stretching bands are dependent on the hydroxylic character of the group, which may suffer reduction through internal or external hydrogen bonding.Thus, a 3-hydroxyl, which can usually be recognized through a

medium to strong band centred between 3350 and 3250 cm^{-1},contributes less towards chelation of the carbonyl in the concomitant presence of a 5-hydroxyl, as shown by a shift of the pertinent band of about +70 cm^{-I}. In spite of this spread in wavelength, the band is diagnostically important (5-OH: very weak, 3000-2500 cm^{-I}; 7-OH: strong, broad, 3200-3100 cm^{-I}; 4'-OH: strong, 3450-3350 cm^{-I}; other: strong, 3500 cm^{-I}).The frequency of the carbony1 stretching is of rather limited significance in the structural study of a flavonol, at least prior to know1edge concerning substitution at C-5. 3-O-Methyl derivatives show spectral characteristics which are closely reminiscent of flavones with identica1 substitution on rings A and B[51].

Table 1.4 Band I to Ia bathochrome $AlCl_3$ -shifts in the UV spectra o! flavonols*

	Shifts (nm) observed in	
	MeOH/HCl or EtOH	MeOH
3-OH-5-H/OH/OR	60 ± 5	
3-OR-5-OH	42 ± 5	
3-OR-5-H/OR	0	
Increments for		
3',4'-diOH	0	35 ± 5
3',4',5'-triOH	0	20
6,7/7,8-diOH	0	10

The procedure distinguishes between C-6 and C-8 oxygenation, since the presence of OH or OMe groups at C-6 produces exceptional values, somewhat higher ones (65-70 nm) for the 3-OH derivatives, and lower on es (20 ± 3 nm) for the 5-OH derivatives[52].

Even in the more favourable case of 3-hydroxylation, however, substitution at C-5 has more effect. Thus the maximal absorption of 3, 5-dihydroxyflavone (1640 cm -I) appears at1616 cm^{-I} in the 3-OH-5-OMe derivative[53]. Furthermore, methylation of the 3-hydroxy1 produces the expected shift (+20 cm^{-I}) to higher wave1ength on1y in the absence of OH or OMe at C-5. The same is true for acety1ation at position 3, the shift being of surprising magnitude (+40 cm $^{-I}$); in contrast acety1ation of a 5-hydroxyl produces a relatively small shift (15 cm $^{-I}$), and this to lower wave length[54] .the above data refer to

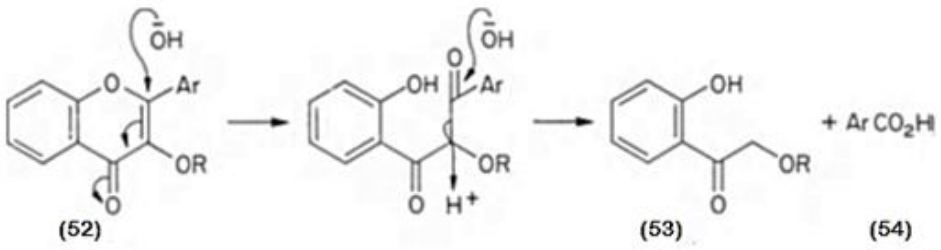
spectra taken in solution. Solid state spectra are affected by molecular interactions and are more erratic. They induce usually, but not always, displacements to lower wavelengths of the OH absorption by about 100 cm^{-1} and of C= O absorption by about 10 cm^{-155}.

The mass spectrometry of flavonols at the very least provides valuable information of structural data obtained by other spectral procedures.

Chemical analyses of naturally occurring flavonols include cleavage reactions and reactions which modify functional groups with the aim of conversion into known derivatives. Such modifying reactions are also essential tools in the partial synthesis of a desired flavonol from another, more readily available, compound.

Only flavonols in which the 3-hydroxyl is free can be fully characterized simply by UV spectrophotometry. Alkaline fusion on the semi-micro scale produces acetophenones. Upon treatment with HBr, 3-hydroxyflavones give α-hydroxyketones which reduce' Tollen's and Fehling's reagents and form osazones. Once a compound is thus recognized as a flavonol, it is usually submitted to alkaline degradation. No other method will reveal the complete structure with such certainty; not even total synthesis, which is frequently be set with ambiguities.In principle, alkaline fissions are retro-grade Allan-Robinson condensations in which the 3-alkoxyflavone (52) c1eaves into 2-

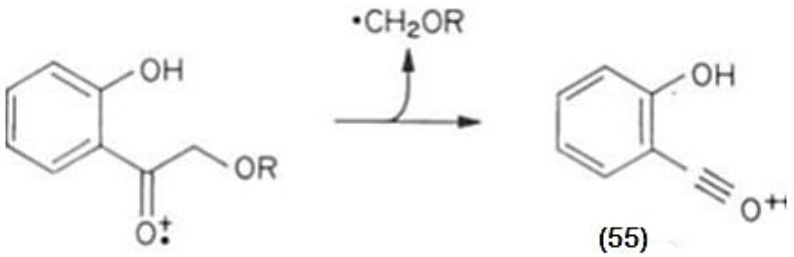
alkoxy-2'-hydroxyacetophenone (53) and benzoate (54). The ease of



Attack of the hydroxyl ion thus depends on the strength of the partial positive charge on C-2. If this is reduced by the presence of an oxide ion at C-3 (created by the strong base from a free 3-hydroxyl), the reaction will only succeed under very vigorous conditions: fusion with KOH or reflux with 40-60% aqueous KOH. Free hydroxyls at C-5, C-7, C-2' and C-4 reduce the activity of the C-4 carbonyl, and compounds with such substitution require conditions of intermediate vigour: i.e. reflux with 20-40% aqueous ethanolic KOH. Clearly then, fully etherified flavonols are most readily cleaved and this is indeed the case: it suffices to reflux them with 5-20% ethanolic KOH[56]. These differences in stability in alkaline medium are of diagnostic value, unless, of course, they are miss interpreted.[57].The benzoic acids originating from the B-rings of flavonols are isolated under most conditions and are easily identified. The situation concerning yield, nature and identification of products derived from ring A is more complex;

indeed, there are a considerable number of publications omitting any mention of these A-ring fragments. If the reaction is carried out on a micro-technique it is usually only possible to detect the B-ring acid by paper chromatography. KOH fusion causes partial de-etherification and yields free phenols from ring A, or else hydroxybenzoic acids. Strong (40-60%) KOH solution, although not affecting the methoxyls, also produces phenols from ring A [58].

However, reasonable yields of the 2-alkoxy-2'-hydroxyacetophenones can only be obtained by using KOH solutions of less than20% concentration. The identification of these acetophenones relies nowadays either on oxidation in pyridine to benzoic acids or on mass spectrometry, the base peak corresponding to the ion (55) indicating the nature of the aliphatic alkoxyl [59].



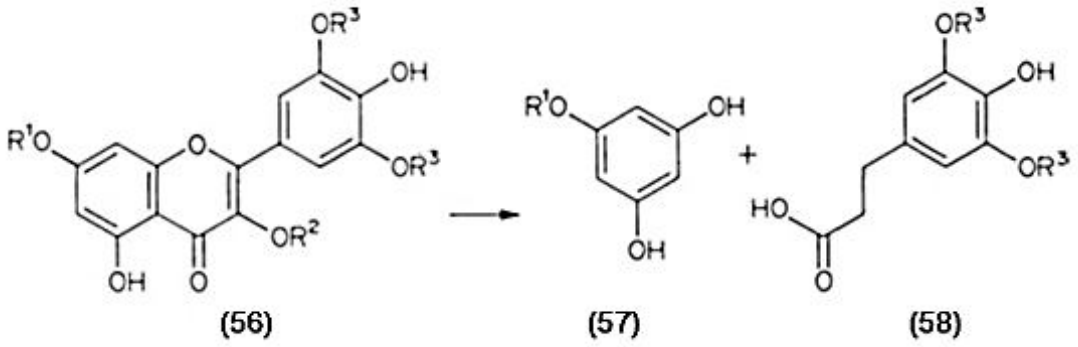
Instructural investigations, data are often derived by degradationswith alkali of the polyhydroxyflavonols and their partially orper-O-methylated derivatives. However the simplest way to deducethe structure is to combine O-ethylation with

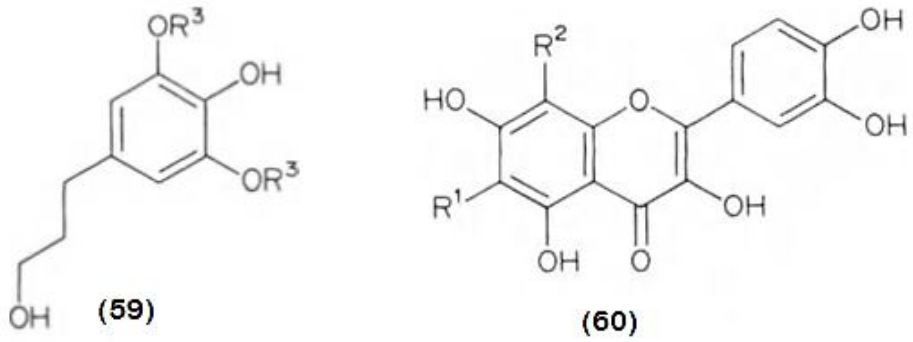
subsequent alkalinefission under the mildest possible conditions[60].

Little, if any, advantage is gained by replacing alkaline degradation with oxidative cleavage. The instability in air of an alkaline solution has long been used for detecting the presence of a 3, 4'-dihydroxy system in a flavonol. The reaction, performed on isorhamnetin on a preparative sca1e, gives phloroglucinol and vanillic acid. Structural analyses of 3, 7-di-O-methylkaempferol and of 3, 5, 6, 7, 4' -pentamethoxyflavone have included oxidation with alkaline peroxide to 4-hydroxybenzoic acid and ozonolysis to anisic acid [61].

By contrast, it is possible that alkaline degradation will in time be complemented, if not replaced, by reductive cleavage. This works Weil on a microscale, a major advantage over the classical process, since the reducing atmosphere engendered by sodium amalgam avoids loss of labile products by oxidation. All classes of flavonoids follow the examples of europetin (56, R1 = Me, R2 = R3 = H), annulatin (56, R1 = R3 = **H,** R2 = me) and syringetin (56, R1 = R2 = **H,** R3 = me) which give the simple phenols (57), phenylpropionic acids (58) and phenylpropanols (59) of expected structure. One warning, however: dehydroxylation of the phenols may occur even under these mild conditions. Thus, while minor amounts of resorcinol accompany the expected phloroglucinol from kaempferol, quercetin and morin, phloroglucinol and pyrogallol, and not 1,

2, 3, 5-tetrahydroxybenzene, are produced from quercetagetin (60, R I = OH, R2 = H) and gossypetin (60, R1 = **H,** R2 = OH) respectively.[62]





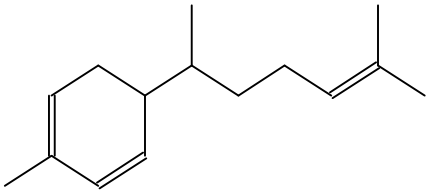
Quercetagetin and gossypetin can additionally be used to illustrateone more method of structure elucidation in the flavonol field; namely reductive acetylation with Zn/ AC_2O in the presence of NaOAc, foIlowed by treatment with HCl/EtOH, leading to 3-hydroxyflavylium salts. In the present case, both flavonols give 6-hydroxycyanidin, since ring isomerization occurs during treatment with acid [63].

1.9. *Zingiber Officinale*

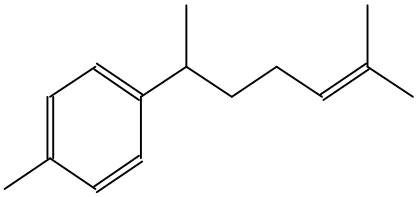
Commonly called ginger belongs to the family Zingiberaceae. The plant is a knotted, thick, beige underground stem (rhizome) that has been used in traditional medicine to aid digestion and treat stomach upset, diarrhea, nausea, and arthritis for centuries. In addition to these medicinal uses, ginger continues to be valued around the world as an important cooking spice and is believed to help the common cold, flu-like symptoms, headaches, and even painful menstrual periods. Today, ginger root is widely used as a digestive aid for mild stomach upset and is commonly recommended by health care professionals to help prevent or treat nausea and vomiting associated with motion sickness, pregnancy, and cancer chemotherapy [64]. Ginger is used as support in inflame matory conditions such as arthritis, and may even be used in heart disease or cancer [64]. Although the beneficial effect of ginger has been exploited, little research has been conducted on its activity on male reproductive functions except a study that reported that Z. Officinale possess androgenic property [64].

Ginger, valued as a spice has been used through ages in almost akk systems of medicine against many ailments. The plant is indogenous to southern Asia and is cultivated in a number of countries including India and Pakistan. The medicinal part of the plant is the roots.

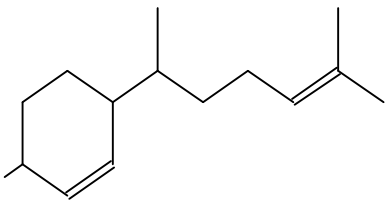
Ginger is a rich source of volatile oils; zingiberol, zingiberrine, phellandrene and linalool are important constituents of the oil. They account for the aroma of the plant. The pungency of the ginger is due to gingerols and shagaols.Investigatons have shown gingerols and shagaols to be mutagenic.In addition, ginger contains special group of compounds called diarylheptanoids including gingerenone [64].



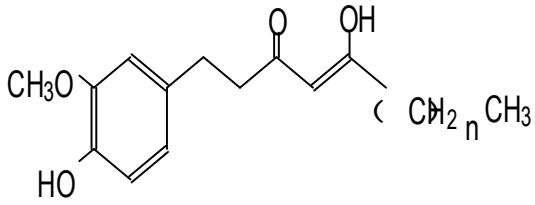
Zingiberene



Cucumene



B-Sesquiphellandrene

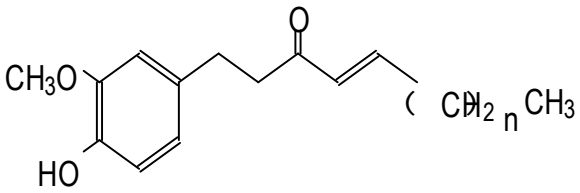


Gingerols

[6]-gingerol; n=4

[8]-gingerol; n=6

[10]-gingerol; n=8



Shagaols

[6]-Shagaol; n=4

[8]-Shagaol; n=6

[10]-Shagaol; n=8

Ginger rhizomes

Ginger

In ethnomedicine the plant is used as carminative, expoctarant and stringent. Recently, a number of studies have been conducted which sowed pharmacological effects of ginger. Some studies have been published about cholesterol lowering effect. The effect of the ethanolic extract of ginger was evaluated in cholesterol-fed rats. The marked rise in serum and tissue cholesterol, serum triglycerides, serum lipoprotein and

phospholipids that followed 10 weeks of cholesterol feeding was significantly reduced by ethanolic extract of ginger. The results were compared with that of Gemfibrozil – a standard orally active hypolipideic drug. The results indicated that ginger is an antihyperlipidemic agent[64].

Cisplatin causes nausea, vomiting and inhibition of gastric emptying. In a study, the acetone and ethanolic extracts of ginger were evaluated for anti-emetic effect in cisplatin – induced emesis in dogs and significant results were obtained. Other studies also indicated the role of ginger in the treatment of nausea and vomiting [64].

Earlier in vitro studies have indicated that aqueous and organic solvent extracts of ginger possess antioxidant and anti-inflammatory potential. In a study, researchers evaluated ethanolic extracts of ginger as anti-tumour agent. It seems that this extract possesses anti –tumour effects in rat skin tumorigensis model. The mechanism of this effect may involve inhibition of tumour promoter-caused cellular, biochemical and molecular changes in rat skin[64].

Rhino viruses are among those viruses which cause the common cold. The dried rhizomes of ginger have been investigated for anti-rhino virus activity. B-sesquiphellandrene- a ginger constituent- showed significant activity[64].

The pharmacological action related to the antimotion sickness effects of ginger was studied. Ginger juice reduced the

spontaneous contrile frequency and enhanced the spontaneous exhibits anticholinergic and antihistaminic action. Ginger produces antimotion sickness action possibly by central and peripheral anticholinergic and antihistaminic effects. Two more studies emphasized the role of ginger juice in the treatment of motion sickness [64].

The inhibiters of prostaglandin biosynthesis are directly related to anti-inflammatory and antiplatelet aggregation activities. The rhizomes of ginger contain potent inhibiters against prostaglandin biosynthesizing enzyme. Gingerols and diarylheptanoids were identified as active compounds [64].

In another study eugenol, present in ginger oil, was shown to be anti-inflammatory agent. Oral administration of augenol cause significant suppression of paw and joint swelling.

A Double- blind randomized crossover trial of the efficiency of ginger in *hyperemeisis gravidarum* was conducted. Powdered of ginger in a daily dose of one gram during four days was effective in diminishing or eliminating symptoms' of *hyperemesis gravidarum* [64].

Aim of this study

This study was aimed to

i) Extraction of ginger phenolic
ii) Isolation of the major flavonoid from the targeted species
iii) Conducting UV studies on the isolated component

2-Materials and Methods

Analytical grade reagent was used. The UV Spectra were recorded on a Perkin-Elmer UV-1800 Spectrophotometer.

2.1-Materials

2.1.1-Collection of plant material

The plant material was collected from Niala – western Sudan and kindly authenticated by the Aromatic Plants Research Institute –Khartoum - Sudan.

2.2-Methods

2.2.1-Preparation of test reagents for phytochemical screening

i)-Flavonoid test reagent

Aluminum chloride solution

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

Potassium hydroxide solution

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

Ferric chloride solution

(0.5g) Ferric chloride was dissolved in (100ml) methanol.

ii) - Alkaloid test reagents

Modified Dragendorffs reagents

Stock Solution (A)

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

Stock Solution (B)

(8g) Potassium iodide were dissolved in (20ml) water.

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

2.2.2- Plant extract

Powdered air-dried of roots of *Zingiber officinale* were macerated at room temperature with 95% ethanol (5 L) for 48h... The solvent was evaporated under reduced pressure to give a solid mass (24g).

2.2.3- Phytochemical screening

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

Test for steroids

Part of the crude product was stirred with petroleum ether to remove most of the colouring malter. 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 .

Test for alkaloids

(5ml) of 2N hydrochloric acid were added to the crude extract (60mg) 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 Dragendorffs reagent were added .No precipitate was formed.

Test for flavonoids

Part of the crude extract (70mg) 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 filtrate, few drops of 1% methanolic aluminum chloride solution were added. Formation of yellow colour indicated the presence of flavonoids.

ii) - To (3ml) of filtrate, few drop of potassium 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.

Test for glycosides

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

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) - Aluminum chloride stock solution

(5g) of fresh anhydrous aluminum 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 sodium acetate was used.

2.2.6- Isolation of flavonoids

Open column (80cm) was used for fractionation the ethanolic etract. Silica gel with particle size 100-200 mesh from LOBA was used.

The composition of the mobile phase (methanol: chloroform in different ratios: 1:4; 2:3 and 2:1; v: v) was determined by TLC analysis. The column was packed with slurry of silica gel with chloroform and then allowed to equilibrate for two hours before use.

The ethanolic extract (5 g) was mixed with 10 g of silica gel G and then applied on the top of the column. Elution commenced by $CHCl_3$: MeOH; 4:1.Then a ratio of chloroform: methanol (3:2) was used followed by a ratio of 2:1(methanol: chloroform) .TLC studies showed that the ratio of 1:4(methanol: chloroform) gave a fraction rich in flavonoids. This fraction was subjected to TLC fractionation using silica gel and the solvent system; methanol: chloroform (1:4). After the usual workup compound I (R_f 0.65) was isolated.

2.2.7- The UV spectrum in presence of UV shift reagents

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.

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.

The UV spectrum in presence of $AlCl_3$/HCl

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

The UV spectrum in presence of sodium acetate

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

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 to give a saturated solution .The UV spectrum was recorded after two minutes.

3- Results and Discussion

3.1-Phytochemical screening

Qualitative tests on the ethanolic extract of *Zingiber officinale* revealed the presence of steroids, flavonoids, glycosides but alkaloids, tannins were not detected (Table 3.1).

Table 3.1: Phytochemical screening of *Zingiber officinale*

Species	Alkaloids	Glycosides	Steroids	Flavonoids	Tannins
Zingiber officinale	**-ve**	**+ve**	**+ve**	**+ve**	**-ve**

3.2- Spectral data of compound I

A Flavonoid- compound I was isolated from the roots of Zingber*officinate*(Ginger) and classified using UV data. The hydroxylation pattern of the isolate was predicted by using some UV shift reagents.

The UV spectrum is considerably enhanced by the use of specific reagents which react with one or more functional groups on the flavonoid nucleus. The addition of each of these reagents separately to an alcoholic solution of the flavonoid induces structurally significant shifts in the UV spectrum. Shifts of this type are commonly induced by the addition of: sodium methoxide, sodium acetate, sodium acetate/boric acid, aluminum chloride and aluminum chloride/hydrochloric acid.

The UV spectra of most flavonoids consists of two major absorption maxima, one of which occurs in the range 240-285 nm (band II) and the other in the range 300-400 nm (band I). In general terms the band II absorption may be considered as having originated from the A-ring benzoyl system and band I from the B-ring cinnamoyl system (see Table 3.2).

Table 3.2: UV absorption of flavones, flavonols, chalcones and aurones

Flavonoid class	Band I	Band II
Flavones	304-350	240-280
Flavonol(3-OH substituted)	330-360	250-280
Flavonols(3-OH free)	350-390	250-280
Isoflavones	shoulder	245-270
Flavanones	shoulder	270-295
Dihdroflavonols	shoulder	270-295
Chalcones	365-390	240-260
Aurones	390-430	240-270

The major flavonoid of the roots of *Zingber officinate* was isolated by column and TLC techniques. In the UV, the isolate – compound I- absorbs at λ_{max} (MeOH) 265 - 400 nm (Fig.3.1).Such absorption is characteristic of aurones.

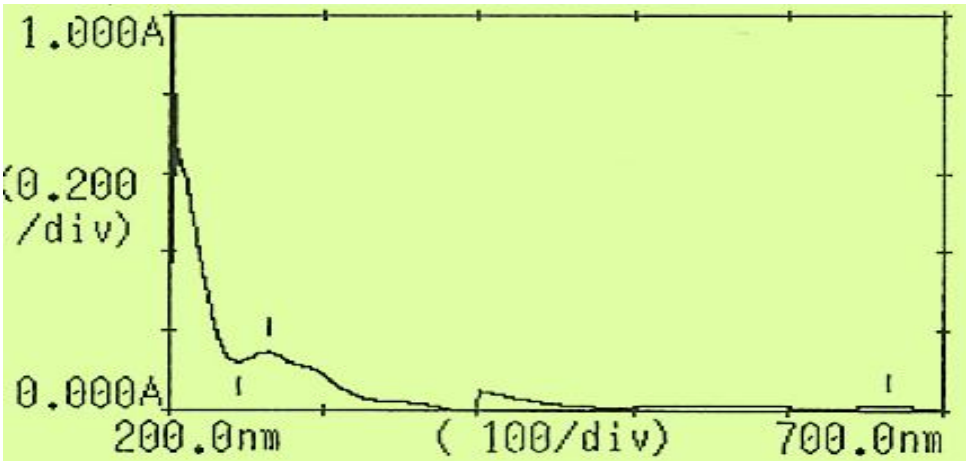


Fig.3.1: UV spectrum of compound I

Sodium methoxide is a strong base and ionizes to some extent all hydroxyl groups on the flavonoid nucleus. However, this shift reagent is used for the specific detection of 3- and 4`-OH functions. In both cases it induces a bathochromic shift, but with decrease in intensity in the case of a 3-OH function.

The addition of sidium methoxide to compound I did not produce (Fig.3.2) any detectable bathochromic shift in all absorption bands, indicating absence of a 3- and 4'-OH groups.

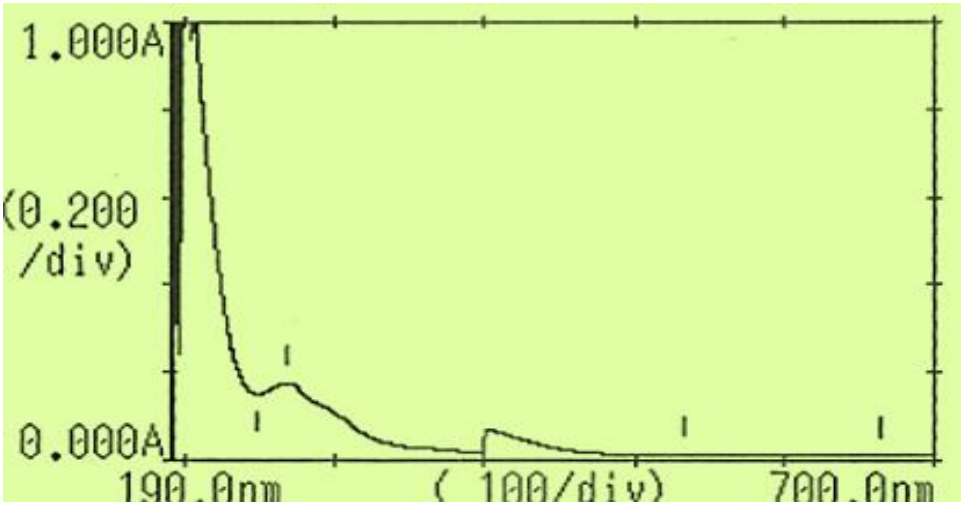


Fig.3.2: Sodium methoxide UV spectrum of compound

Sodium acetate ionizes only the more acidic hydroxyl groups i.e., the 3, 7- and 4'-hydroxyl groups. Because ionization of the 7-hydroxyl group mainly affects Band II (whereas ionization of the 3- and/or 4'-hydroxylgroups mainly affects Band I), sodium acetate is a particularly useful diagnostic reagent for the specific detection of 7-hydroxyl groups.

The sodium acetate spectrum (Fig.3.3) did not give any bathochromic shift, indicating absence of a 7-OH group.

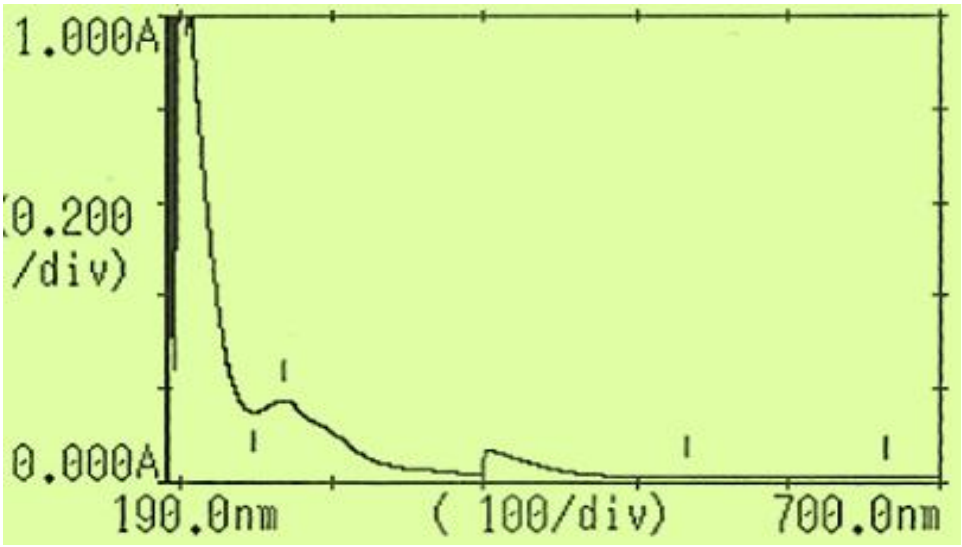


Fig.3.3: UV Spectrum of compound I in sodium acetate

A mixture of sodium acetate and boric acid is used for the detection of o-dihydroxyl groups in all flavonoids. The boric acid spectrum (Fig.3.4) did not reveal bathochromic shifts indicating the absence of catechol systems.

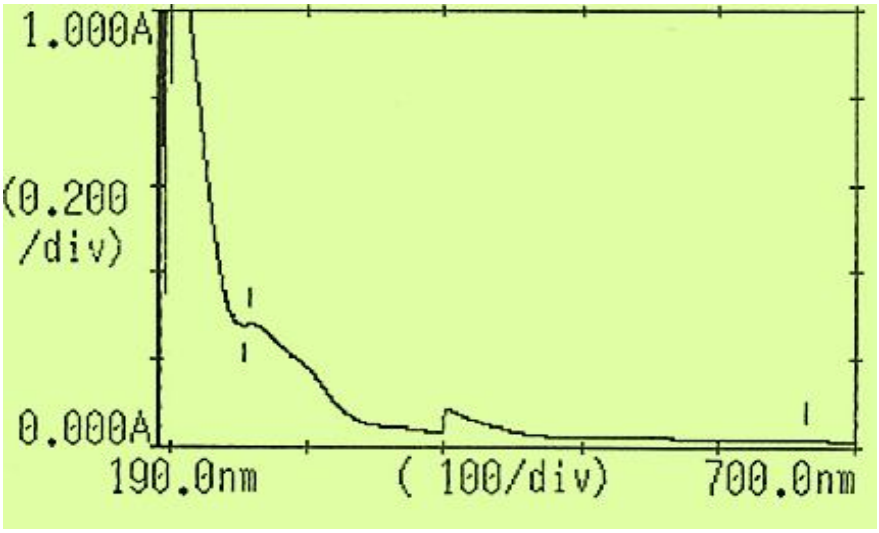


Fig.3.4: UV spectrum of compound in NaOAc+H_3BO_3

Aluminum chloride chelates with functional groups such as: the 5-hydroxy-4-keto-, 3-hydroxy-4-keto-and O-dihydroxy1 systems, and this reaction is evidenced by bathochromic shifts of one or both bands in the spectrum.

When $AlCl_3$ was added to a methanolic solution of compound I, no bathochromic shift was observed (Fig.3.5) indicating absence of 3- , 5-hydroxyl groups as well as catechol moieties.

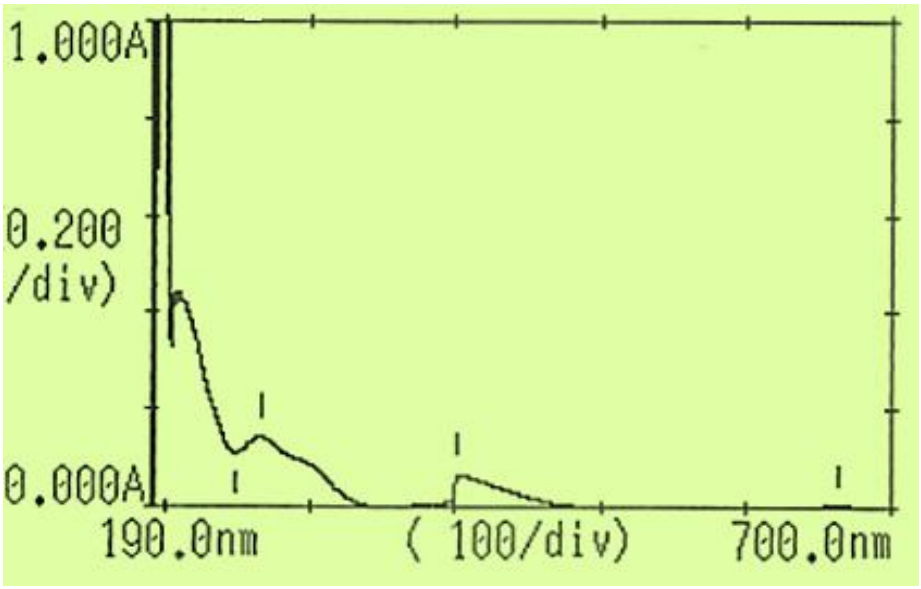
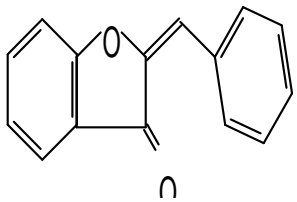


Fig.3.5: The aluminum chloride spectrum of compound

The above UV data suggest a pattern characteristic of aurones. However, the isolated aurone is not hydroxylated at C_3, C_5, C_7 or $C_{4'}$, also it lacks catechol systems.



Aurone

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