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ISOLATION AND PARTIAL STRUCTURE OF A DIHYDROCHALCONE FROM ACACIA MELIFERA (FABACEAE- MIMOSOIDEAE)

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ABSTRACT

Acacia melifera – Known locally as "Kitir" – is a commonly occurring plant throughout the savannah in southern, eastern and western Africa. The plant has many potential medical benefits and is widely used in African system of medicine. In this study a methylated dihydrochalcone was isolated from bark and its structure was partially elucidated via a combination of spectral techniques (UV-Vis. and ^{1}HNMR and MS).

KEYWORDS: Acacia melifera, Isolation, Dihydrochalcone, Partial structure.

INTRODUCTION

The genus Acacia, which comprises more than 1200 species, is the second largest genus in the family Fabaceae- Mimosoideae. Acacia melifera – Known locally as "Kitir" – is a commonly occurring plant throughout the savannah in southern, eastern and western Africa. The gum from injured stems is edible and leaves are grazed by animals.[1-10] The plant was screened for major secondary metabolites and different bioactive constituents have been reported including: flavonoids, tannins, saponins, tepenoids and alkaloids.[11-13]

Bark is used traditionally against malaria, syphilis, bowel disorders and cold.[14,15] The antimicrobial activity of Acacia melifera has been documented.[16] It has been reported that Acacia melifera possesses antiviral potency against (HIV-1) and Herpes simplex.[17] The hepatoprotective and anti-HBV have been reported.[18]

With this background on the beneficial attributes of Acacia melifera, it was decided to investigate the flavonoids of this species which are well known for their health promoting effects mainly through their potent free radical scavenging capacity.

MATERIALS AND METHODS

Materials

Plant material

Barks of Acacia mellifera were collected from Nyala – western Sudan.The plant was authenticated by Dr. Abdel Halem, Department of Forest, College of Agriculture, University of Bahri, Sudan The freshly collected plant material was dried under shade at room temperature, cut into small pieces and powdered.

Materials for chromatographic study

- Whatman paper No.1 (1mm) for paper chromatography (Whatman Ltd. Maistone, Kent, England).
- Whatman paper No (3mm) for preparative paper chromatography (Whatman Ltd. Maistone, Kent, England).

Equipments

1- Ultra - Violet - Visible spectrophotometer (Shimadzu model UV240 and 240PC).
2- Joel- Nuclear Magnetic Resonance (NMR) spectrophotometer (Brucker AC-250) -500 MHz.

Extraction and isolation of flavonoids

(2Kg) of Acacia mellifera barks were extracted with 95% ethanol for 10 h. at 60 ^{o}C in a Soxhlet apparatus. The extract was filtered and the solvent was removed under reduced pressure. A sequential solvent extraction using a number of solvents (chloroform, ethyl acetate and n-butanol) of varying polarity was used for the preliminary separation of flavonoids.

The ethyl acetate fraction - being rich in phenolics- was applied as a streak on 20 sheets of Whatman 3mm paper (46×57 cm) and run in solvent (BAW:6:1:5;v:v:v) over night. The dried papers were viewed and examined under visible and ultraviolet light, then exposed for 2-3 minutes

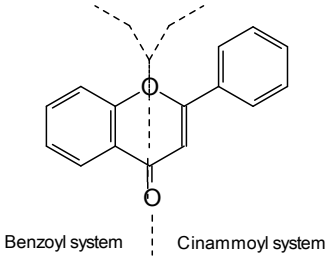
to ammonia vapor and immediately re-examined to observe changes in color of fluorescence. Chromatograms were located under UV light by pencil, cut off and similar bands were joined and cut into small pieces. A flavonoid was eluted from paper by methanol after several hours of contact. Evaporation of the solvent under reduced pressure gave a chromatographically pure flavonoid- compound I.

RESULTS AND DISCUSSION

Identification of compound I

The structure of compound I- which was isolated from Acacia melifera barks - was partially elucidated via a combination of two spectral techniques (UV-Visible and ^{1}HNMR spectroscopy).

Flavonoids usually exhibit two absorption bands in their UV spectra; band I and II. Band I is associated with the absorption of the cinnamoyl system, while band II originates from the benzoyl system. Flavones, flavonols, chalcones and aurones give both bands (I and II), due to conjugation between the carbonyl function and the aromatic B ring. While flavanones, isoflavones, dihydroflavonols and dihydrochalcones afford only band II in the range: 230-290nm.These classes of flavonoids lack conjugation between the B ring and the carbonyl function.



In the UV compound I absorbs (Fig.1) at λ_{max} 207, 275nm.Such absorption is characteristic.[19] of: isoflavones, flavanones, dihydrochalcones and dihydroflavonols. However, isoflavones are characterized by a shoulder.[19] in the range: 300-340nm and such shoulder was not detected in the spectrum. Furthermore, the UV shift reagent-sodium methoxide- did not reveal any bathochromic shift in the spectrum (Fig.2).This indicates absence of a 3-OH group which is characteristic of dihydroflavonols. Sodium methoxide is a strong base and is used for the specific detection.[19] of 3- and 4`-OH functions. In both cases it gives a bathochromic shift, but with decrease in intensity in case of a 3-OH function.[19]

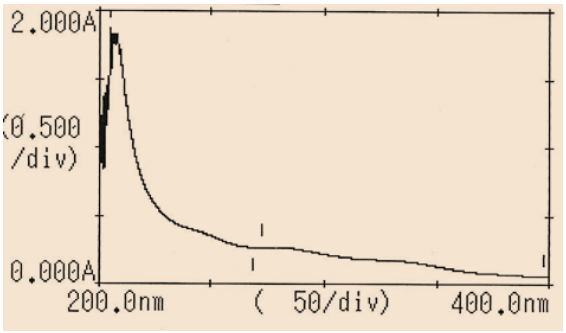


Fig. 1: UV spectrum of compound I.

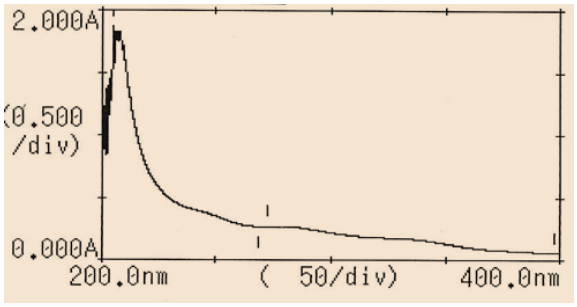


Fig. 2: Sodium methoxide spectrum of compound I.

The ^{1}HNMR spectrum can differentiate[19] between flavanones and dihydrochalcones. The magnetically unequivalent C_3-protons split each other into a double doublet which is further splited into a double quartet (usually merge into a pair of multiplets) by C_2 – protons. Hence flavanones are characterized by two multiplets around δ2.8 and δ5.2ppm.Such multiplets were not observed in the ^{1}H NMR spectrum (Fig.3) of compound I, and this compound is thus a dihydrochalcone. The ^{1}HNMR spectrum revealed a signal at δ0.85 (3H) assigned for a methyl group. The resonance at δ1.24 was assigned for the two methylene groups of the dihydrochalcone moiety. The A ring protons resonated at higher field relative to the B ring protons at δ6.70 due to the deshielding influence of the heterocyclic C ring on the neihgbouring B aromatic ring. The signal at δ7.04 account for B ring protons (The solvent –DMSO- residual protons appear around δ2.50, while the residual water protons appear around δ3.30).

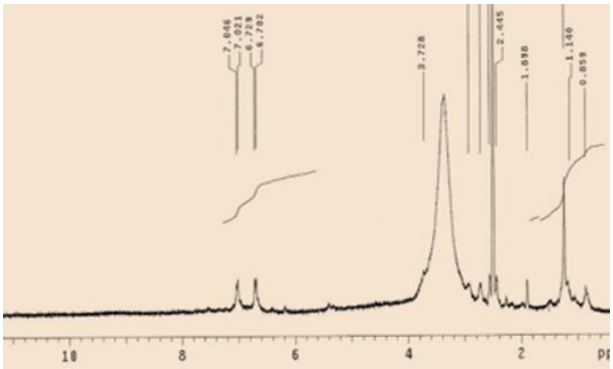


Fig. 3: ^{1}H NMR spectrum of compound I.

The hydroxylation pattern of the dihydrochalcone was investigated via the UV shift reagents[19] sodium acetate,

aluminium chloride and boric acid. The sodium acetate spectrum did not reveal any bathochromic shift indicative of a 7-OH function (Fig.4).

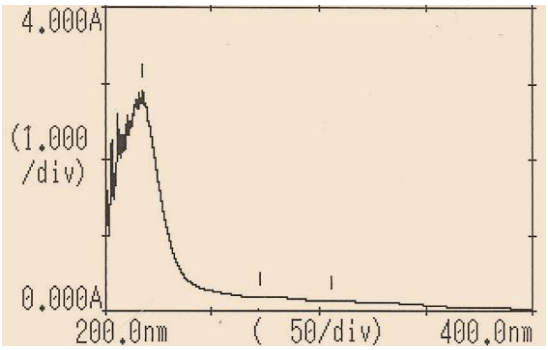


Fig. 4: Sodium acetate spectrum of compound I.

No bathochromic shift was detected in the aluminium chloride spectrum and this is suggests absence of a 3- and 5-OH functions as well as catechol moieties in both aromatic rings (Fig.5).The same trend was observed in the boric acid spectrum (Fig.6) which is diagnostic of catechol systems.

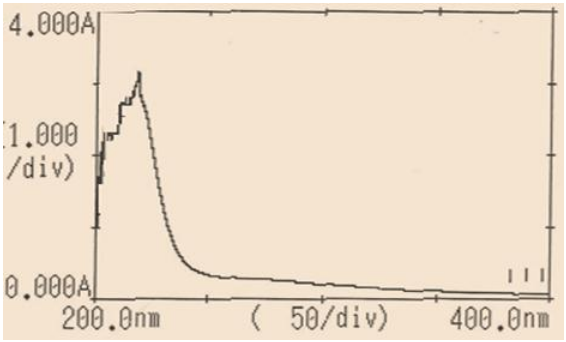


Fig. 5: Aluminium chloride spectrum of compound I.

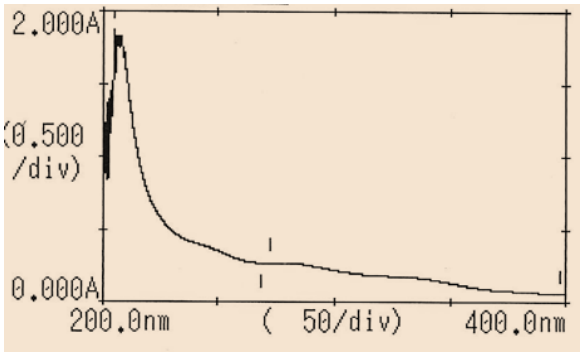
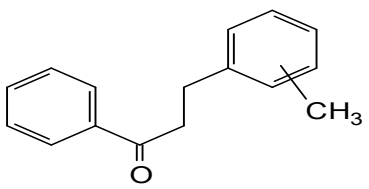


Fig. 6: Boric acid spectrum of compound I.

On the basis of the above cumulative data the following partial structure was proposed for compound I:



Compound I

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