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ISOLATION OF A FLAVONE FROM LEAVES OF *ARISTOLOCHIA BRACTEATA* LINN. (ARISTOLOCHIACEAE) GROWN IN SUDAN

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ABSTRACT

This study was set to investigate the flavonoids of *Aristolochia bracteata* which is a key species in indigenous medicine. Flavonoids encompass a large group of polyphenolic substances with marked physiological potential including: antibacterial, anti-inflammatory, antiallergic, antifungal, antimutagenic, antiviral and vasodilator effects. *Aristolochia bracteata* showed many beneficial pharmacological effects. It is used in ethnomedicine as anthelmintic, antiinflammatory, antipyretic and as purgative. Roots are used by local healers to treat gonorrhea and syphilis. In this study a flavone was isolated from leaves by paper chromatography and its structure was partially elucidated on the basis of its spectral data(UV,^{1}HNMR and MS).

KEYWORDS: Aristolochia bracteata, Isolation, Flavone, Partial Structure.

INTRODUCTION

Flavonoid compounds are phenolics which are widely distributed in plants.[1-4] The basic skeleton of flavonoids is based on a fifteen carbon atoms , arranged in a C_6-C_3-C_6 configuration consisting of two aromatic rings (A and B) linked by a three carbon unit which may or may not form a third heterocyclic ring (C). Major groups of flavonoids include: flavones, flavonols, chalcones, aurones, flavanones, isoflavones, dihydroflavonols, dihydrochalcones, anthocyanins and flavans. Flavonoids encompass a large group of polyphenolic substances with marked physiological potential including: antibacterial, anti-inflammatory, antiallergic, antifungal, antimutagenic, antiviral and vasodilator effects.[5-7]

Aristolochia bracteata is a plant of many medicinal attributes in the family Aristolochiaceae. The herb is used in ethnomedicine as anthelmintic, antiinflammatory, antipyretic and as purgative. Roots are used by local healers to treat gonorrhea and syphilis. *Aristolochia bracteata* showed many interesting pharmacological effects. Leave aqueous extract produced positive ionotropic effect on heart of model animals. The extract also showed a wide therapeutic index. The mechanism underlying the positive ionotropic effect was also addressed.[8]

It has been reported that *Aristolochia bracteata* extracts exhibited significant antipyretic activity.[9] The chloroform extract showed potent antiallergic activity in experimental models.[10] *In vivo* studies testified that regular treatment of model animals with *Aristolochia bracteata* extracts significantly improved ESR and Hb value.[11] It has been shown that the ethanol extract of *Aristolochia bracteata* exhibited significant antibacterial activity against a panel of human pathogens.[12] *Aristolochia bracteata* showed significant free radical scavenging capacity.[13] Such results lend a rationale for the anti-inflammatory activity of this species.In carrageenan- induced paw edema, the ethanol extract of the leaves demonstrated significant reduction in edema volume.[14] Also it has been reported that the chloroform fraction of *Aristolochia bracteata* gave promising trypansocidal effect.[15] Extracts of *Aristolochia bracteata* exhibited significant antifungal activity against some standard fungi.[16] The aqueous extract of this species exhibited significant antiulcer effect in ethanol-induced ulcer.[17] The wound healing, antiangiogenetic and abortifacient properties were also reported.[18,19]

MATERIALS AND METHODS

Materials

Plant material

The leaves of *Aristolochia bracteata* were collected from Nyala (western Sudan). The plant was identified and authenticated by direct comparison with a reference herbarium sample.

Instruments

A multiband UV λ_{max} (254 / 365 nm) portable ultraviolet lamp (6 watt S/Y and L/W) was used for viewing chromatograms in paper chromatography. Ultraviolet absorption spectra were obtained in spectroscopic methanol on UV -Visible Spectrophotometer (Shimadzu).

^{1}HNMR spectra were run on a Bruker AM 500 spectrophotometer (Germany) operating at 500 MHz in spectroscopic grade DMSO-d_6. The chemical shifts values are expressed in δ (ppm) units using (TMS) as an internal standard.

Methods

Isolation of flavonoids

Powdered leaves of *Aristolochia bracteata* were macerated with 95% ethanol at room temperature for 48 hours. The solvent was removed under reduced pressure to give a crude extract. The crude extract was dissolved in the minimum amount of ethanol and was applied on Whatman papers (No. 3 mm) as narrow strips. The bands were irrigated with 15% acetic acid. The developed chromatograms were air-dried and examined under both visible and UV light (λ_{max} 366,245nm). The equivalent bands from each paper were then cut out, combined and cut into small strips and slurred with methanol. After several hours of contact, with occasional shaking, the solvent was evaporated *in vacuo* to dryness. In this way- a flavonoid- compound I was isolated in chromatographically pure form.

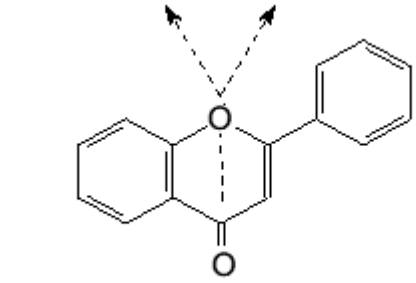
RESULTS AND DISCUSSION

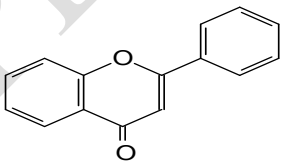
Phytochemical screening

Phytochemical screening of *Aristolochia bracteata* leaves revealed the presence of alkaloids, flavonoids, steroids, terpenoids, tannins and saponins.

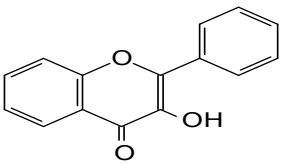
Characterization of compound I

In their UV spectra flavones,flavonols,chalcones and aurones give both band I(due to cinnamoyl chromophore) and band II(due to benzoyl chromophore). Other classes: isoflavones, flavanones, dihydrochalcones and dihydroflavonols show only one peak(Band I) originating from the benzoyl system. Band I, usually 300 – 400nm and band II usually 240 – 290 nm.[1,2]

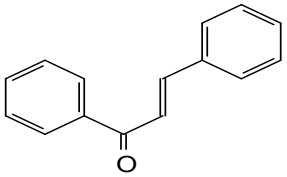




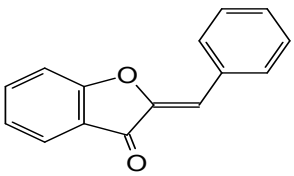
Flavone



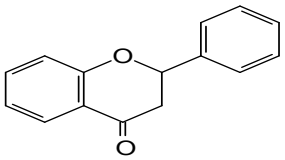
Flavonol



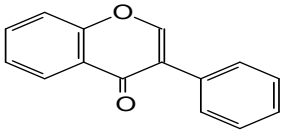
Chalcone



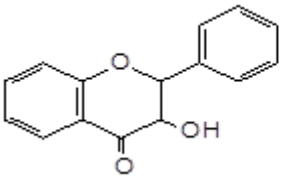
Aurone



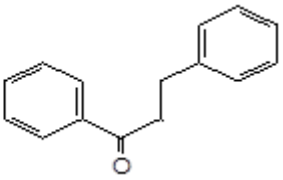
Flavanone



Isoflavone



Dihydroflavonol



Dihydrochalcone

Compound I absorbs in the UV(Fig.1) at λ_{max} (MeOH) 285,327 nm.Such absorption is given by flavones.[1,20]

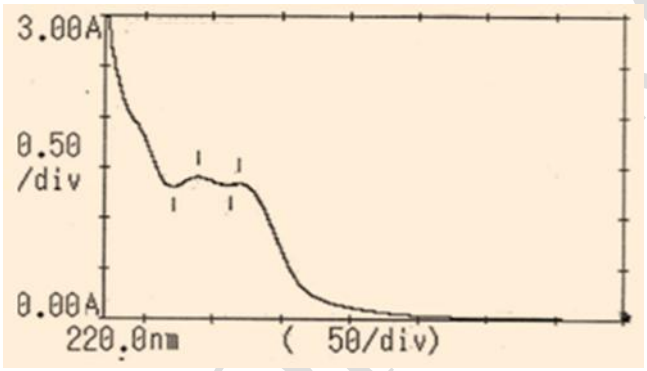


Fig. 1: UV spectrum of compound I.

Very significant structural features have been obtained by utilizing the so- called UV shift reagents which produce shifts in the UV absorption maxima in accordance with the location of the various hydroxyl functions in the flavonoid nucleus[20] ; these reagents are : sodium methoxide (which is diagnostic of 3- and 4`-OH functions);sodium acetate (diagnostic of 7-OH function); aluminium chloride (diagnostic of 3- , 5-OH and catechol systems) and boric acid (diagnostic of catechol systems).

The sodium methoxide spectrum of compound I gave a bathochromic shift characteristic[20] of a 4`-OH group(Fig.2).

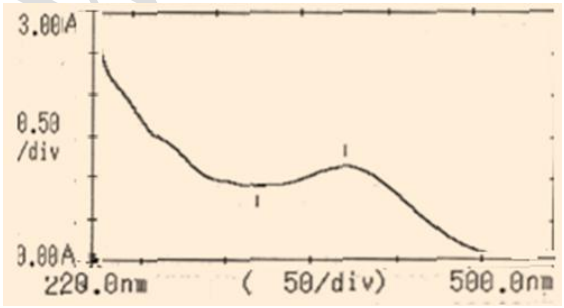


Fig. 2: Sodium methoxide spectrum of compound I.

When sodium acetate was added to a methanolic solution of compound I, no bathochromic shift diagnostic[20] of a 7-OH function was observed (Fig. 3).

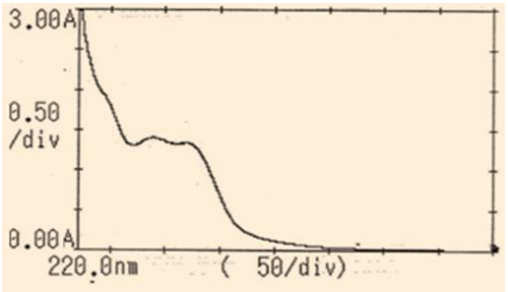


Fig. 3: Sodium acetate spectrum of compound I.

Other shift reagents -boric acid (Fig.4), aluminium chloride (Fig,5)- failed to give any detectable bathochromic shifts. The boric acid spectrum thus suggests absence of catechols systems, while the aluminium chloride spectrum indicated absence of 3- and 5-hydroxylation.

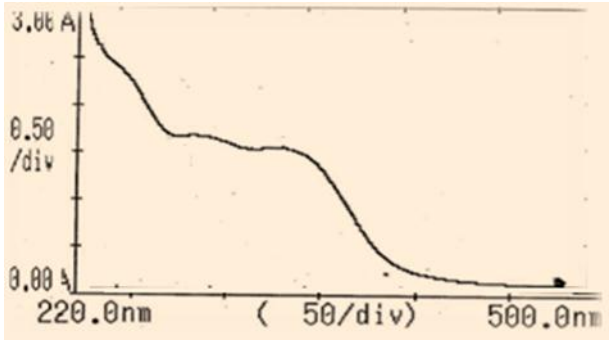


Fig. 4: The boric acid spectrum of compound I.

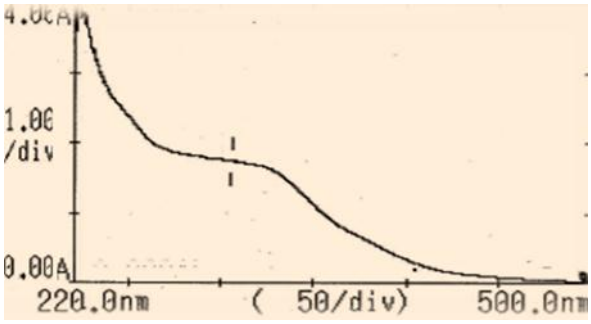
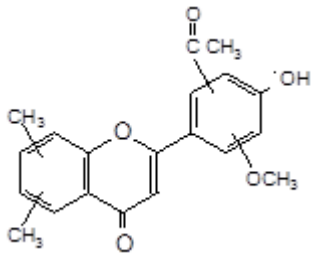


Fig. 5: The aluminium chloride spectrum of compound I.

The [1]HNMR spectrum (Fig. 6) showed: δ1.34(6H) assigned for 2 methyl functions; δ1.96(3H) assigned for an acetyl group ; δ3.40-4.00(m) attributed for sugar protons(not identified in this study); δ4.04 accounting for a methoxyl function . The aromatic protons appeared as multiplets centered at δ6.30, δ6.80, δ7.65 and as singlet at δ7.10ppm.Signals at δ2.5 and δ3.30 are due to solvent (DMSO) residual protons and residual water respectively. On the basis of the above cumulative data, the aglycone of compound I was assigned the following tentative structure:



Aglycone of compound I

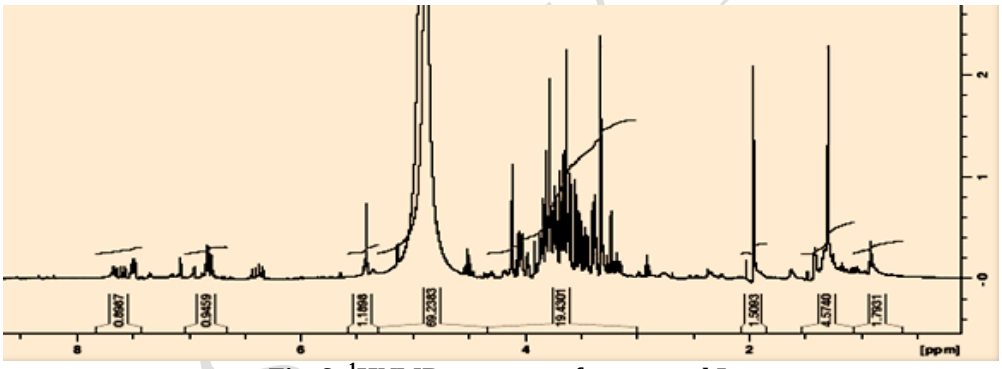


Fig. 6: [1]HNMR spectrum of compound I.

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