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Phytochemical Investigation of *Dalbergia rubiginosa* Stem

M. Jansirani[1,*], R. Gandhidasan[2]

[1]*Department of Chemistry, Govindammal Aditanar College for Women, Tiruchendur – 628 215, TN, India.*
[2]*Department of Natural Products Chemistry, Madurai Kamaraj University, Madurai – 627 017, TN, India.*

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ABSTRACT

The structural determination of two compounds viz., nitiducarpin and dalbergin which are isolated from the benzene and chloroform extract of the stem of *Dalbergia rubiginosa* is discussed in detail. The structures of these compounds are established from the colour reaction, UV, IR, ^{1}H NMR and ^{13}C NMR spectra. In case of dalbergin, the structure is confirmed by preparing the acetate, recording their spectra and comparing the values with the literature values. The structures of nitiducarpin and dalbergin are confirmed by comparison with an authentic sample also.

1. Introduction

The genus *Dalbergia* belonging to the family Leguminosae and sub family Papilionaceae consists of trees, shrubs and woody climbers. This genus comprises over 120 species with a worldwide distribution, out of which about 35 species are known to occur in India [1]. Several *Dalbergia* species are of much economic importance [2]. The genus *Dalbergia* is known to rich sources for isoflavonoids and neoflavonoids [3]. Many *Dalbergia* species like *D. sissoo, D. nigra, D. lanceolaria, D. parviflora* and *D. variabilis* are reported to possess medicinal properties [4]. Species like *D. sissoo, D. latiflolia, D. lanceolaria,* and *D. paniculata* are valued for their good timber. The leaves of *D. lanceolaria* are used in the treatment of arthritic disorders. The extract of *D. hupeana* has been found as a Chinese folk medicine, as an antiscebietic. The extract of the leaves of D. *volubilis* showed significant anti-inflammatory and anti-arthritic activities in rats [5]. The extract of the stem of D. *coromandeliana* showed significant anti-bacteria activity [3]. Similarly the polyphenolic constituents present in *D. sissoides* exhibit anti-inflammatory activities [6].

A number of reviews on *Dalbergia* species have reported. Chemical investigation has been carried out on the following new *Dalbergia* species. D. *canadanatensis,* D. *monetaria,* D. *odorifera*, D. *sissoides*, D. *stipulacea* and D. *malabarica*. New isoflavonoid compounds isolated from the above new species as well as species which have been earlier investigated are accounted [7].

The examination of the nature of the compounds isolated from *Dalbergia* species revealed that mostly they fall into isoflavonoids, flavonoids, neoflavonoids, benzofurans, benzophenones, sterols and terpenoids [8].

2. Experimental Methods

2.1 Plant Material

The stem *Dalbergia rubiginosa* was collected from Pachamalai Hills near Trichy in Tamil Nadu, India. The identified plant species was confirmed by Fr. Dr. K.M. Matthew S.J., Herbarium director, The Rapinat Herbarium Trichy.

2.2 Extraction of the Stem of Dalbergia rubiginosa

The stem of *Dalbergia rubiginosa* was cut into small pieces and dried well. These dried chips (2.5 kg) were extracted successively by refluxing with benzene (4 x 3 litres) and ethanol (4 x 3 liters) respectively. The extract was collected, concentrated and investigated by column chromatographic separation.

Melting points were determined in sulphuric acid bath and are uncorrected. Fourier Transform Infra-Red (FTIR) spectroscopy with Bruker IFS 66 V spectrometer was performed. UV spectra were recorded for using Shimadzu UV-Visible spectrophotometer. ^{1}H NMR spectra in $CDCl_3$ on a Joel GSX Shimadzu 400 MHz spectrophotometer with TMS as an internal standard. ^{13}C NMR spectra in $CDCl_3$ at 300 MHz using TMS as an internal reference.

2.3 Investigation of the Benzene Extract

The benzene extract of the stem on concentration under reduced pressure gave a viscous greenish pasty mass (13 g). It was found to be soluble in ethanol, acetone and chloroform. It was found to be homogenous on TLC. Hence, the pasty mass was subjected to column chromatographic separation. The crude mass was subjected to the following colour reactions. An alcoholic solution of the crude mass with neutral ferric chloride solution gave light green colour indicating the presence of compounds with chelated hydroxyl group. A small amount of the crude mass in chloroform gave red colour with concentrated sulphuric acid suggesting the presence of steroids. A chloroform solution of the crude mass did not produce green colour with a mixture of acetic anhydride and concentrated sulphuric acid indicating the absence of terpenoids. An alcoholic solution of the crude mass with magnesium powder and concentrated hydrochloric acid did not give any characteristic colour suggesting the absence of flavonoids. An alcoholic solution of the crude mass with sodium amalgam and concentrated hydrochloric acid gave no colour showing the absence of isoflavonoid compounds in the extract.

2.3.1 Chromatographic Separation

The crude mass (6 g) was dissolved in minimum amount of acetone and made into a slurry with silica gel (13 g) and transferred onto a column of silica gel (150 g) built in benzene. Elution of the column was carried out successively by using pure as well as mixture of solvents in the order of benzene, chloroform, chloroform-ethanol with different proportions. Fractions of 100 mL were collected each time and distilled. The homogeneity of the fractions was examined by TLC. TLC examination of

*Corresponding Author
Email Address: jansirani.m2011@gmail.com (M. Jansirani)

these fractions was carried out on a number of silica gel plates using different solvent system. Viz.,

I. Benzene – hexane (8 : 2)
II. Pure benzene
III. Pure chloroform
IV. Chloroform – ethyl acetate (9 : 1)
V. Chloroform – ethanol (9.9 : 0.1)

Fractions revealing identical TLC behavior were mixed together.

2.3.2 Compound I (Nitiducarpin)

Compound I crystallized from methanol as colourless fine crystalline solid. It was found to be homogeneous on TLC using various solvents, m.p. 108 °C. It was found to be soluble in ethanol, chloroform and acetone at ordinary temperature. It gave bluish green colour with gallic acid and concentrated sulphuric acid (Labat Test) [9]. It gave red colour with concentrated sulphuric acid. Yield: 90 mg.

Spectral Data

UV λ $CHCl_3$ (nm) : 310, 262.
IR λ_{max}^{KBR} (cm^{-1}) : 1619, 1469, 1382, 1334, 1220, 1178, 1135, 1091, 1041, 931, 871 and 819.
^{1}H NMR ($CDCl_3$) (TMS) (δ ppm) : 1.38 (s, 3H, 2'-CH_3), 1.66, 1.69 (s, 6H, gem-Me_2), 1.71-2.22 (2m, 4H, H-5'), 3.61 (m, 1H, H-6a), 3.65 (t, 1H, H-6ax), 4.26 (dd, 1H, H-6eq), 5.16 (m, 1H, H-7'), 5.51 (d, 1H, H-11a), 5.54 (d, 1H, H-3'), 5.90 (d, 2H, O-CH_2–O), 6.43 (s, 1H, H-10), 6.53 (d, 1H, H-2), 6.67 (d, 1H, H-4'), 6.72 (s, 1H, H-7), 7.24 (d, 1H, H-1).
^{13}C NMR ($CDCl_3$) (TMS) (δ ppm) : 154.753 (C-4a), 154.682 (C-10a), 148.533 (C-3), 148.456 (C-9), 142.035 (C-8), 132.125 (C-8'), 131.087 (C-3'), 128.572 (C-1), 124.447 (C-7'), 118.370 (C-6b), 117.317 (C-4'), 112.440 (C-1a), 110.755 (C-2), 110.427 (C-4), 105.121 (C-7), 101.602 (C-12), 94.227 (C-10), 78.915 (C-2'), 77.839 (C-11a), 66.949 (C-6), 40.525 (C-5'), 41.453 (C-6a), 26.763 (C-6'), 26.073 (C-2'), 18.029, 23.073 (gem dimethyl)

2.4 Investigation of the Alcohol Extract

The alcohol extract on concentration gave a viscous brown pasty mass. It was found to be soluble in chloroform and acetone. An alcoholic solution of the crude mass produced light green colour indicating the presence of compounds with chelated hydroxyl group.

2.4.1 Chromatographic Separation

The viscous pasty mass (9 g) was dissolved in minimum amount of acetone and made into a slurry with silica gel (15 g) and transferred onto a column of silica gel (150 g) built in pure chloroform. The column was successively eluted with solvents on increasing polarity. Fractions of 100 ml were collected each time, distilled and the homogeneity of the fractions was examined by TLC. Fractions revealing identical TLC behavior were mixed together.

2.4.2 Compound II (Dalbergin)

Compound II crystallized from methanol as light yellow colour solid, m.p. 216-218 °C. It was found to be homogenous on TLC using various solvents. It was found to be soluble in ethanol, acetone and chloroform. It gave a negative test with alcoholic ferric chloride and sodium amalgam and concentrated hydrochloric acid.

Spectral Data

UV λ $CHCl_3$ (nm) : 316, 251.
IR λ_{max}^{KBR} (cm^{-1}) : 3240, 1670.
^{1}H NMR ($CDCl_3$) (TMS) (δ ppm) : 3.99 (s, 3H, -OCH_3), 5.61 (s, 1H, -OH), 6.99 (s, 1H, H-5), 7.48 (s, 5H, C-4) (phenyl ring protons).
^{13}C NMR ($CDCl_3$) (TMS) 300 MHz (δ ppm) : 161.036 (C-2), 155.316 (C-4), 149.586 (C-6), 148.852(C-8a), 141.931 (C-7), 135.090 (C-1'), 129.098 (C-4'), 128.358 (C-3', C-5'), 127.845 (C-2', C-6'), 112.065 (C-5), 110.840 (C-4a), 110.025 (C-8), 95.103 (C-3), 55.982 (O-CH_3).

2.4.3 Dalbergin acetate

Dalbergin (20 mg) in dry pyridine (1 mL) was treated with acetic anhydride (0.5 mL). The mixture was kept at room temperature for 24 hours and then poured into crushed ice. The acetate that separated was filtered and dried. It was crystallized from methanol; M.p. 148-150 °C and Lit. m.p. 156 °C.

Spectral Data

^{1}H NMR ($CDCl_3$) (TMS) (δ ppm) : 2.28 (s, 3H, -$OCOCH_3$), 3.99 (s, 3H, CH_3), 6.27 (s, 1H, H-3), 6.98 (s, 1H, H-8), 7.14 (s, 1H, H-7), 7.45 (s, 5H, C-4 phenyl ring protons).

3. Results and Discussion

3.1 Investigation of the Benzene Extract

The benzene extract of the stem on concentration under reduced pressure gave a viscous greenish pasty mass. The crude mass was adsorbed on silica gel and chromotographed on a silica gel, column built in benzene and eluted with solvents of increasing polarity. Benzene elute gave a homogenous colourless solid, compound I (Nitiducarpin). No other compound was isolated from this benzene extract.

3.1.1 Compound I (Nitiducarpin)

The compound I isolated from the benzene eluate of the column, crystallized from methanol as colourless fine crystalline solid. It homogeneity of the compound was ascertained by TLC using various solvents, m.p. 108 °C. It produced no colour with alcoholic ferric chloride solution indicating the absence of chelated hydroxyl group in the compound. It did not answer the tests for isoflavones, isoflavanones and terpenoids. Compound I gave red colour with a few drops of concentrated sulphuric acid. It gave bluish green colour with gallic acid and concentrated sulphuric acid (Labat Test) [9]. This indicates the presence of methylenedioxy group in compound I.

The UV-spectrum of compound I in chloroform exhibited absorption maximum at 310 nm which corresponds to the λ_{max} value of pterocarpans [10]. From colour reactions and UV spectral value, the nature of compound I was inferred as pterocarpan.

The IR spectrum of the compound I indicated the absence of any hydroxyl and carbonyl groups but showed the presence of ether functional group at 1135 cm^{-1} in the compound [11].

The ^{1}H NMR spectrum of the compound in $CDCl_3$ exhibited a number of signals both in aliphatic and aromatic regions. The presence of three singlets at 1.38, 1.58 and 1.66 δ integrating to a total of nine protons revealed the presence of three methyl groups in the molecule. A doublet of doublet at 4.26 δ and a doublet at 5.47 δ each integrating to one proton are corresponding to that of H-6eq and H-11a protons of pterocarpan respectively [12]. The presence of a triplet at 3.65 δ and multiplet at 3.61 δ integrating to one proton are due to H-6ax and H-6a protons of pterocarpans respectively. The methylenedioxyl substituent was placed at C-8 and C-9 position of D-ring and two singlets at 6.72 and 6.52 δ each integrating to one proton were assigned to H-7 and H-10 protons. The presence of three methyl substituents and other signals in the aromatic and aliphatic regions suggested the presence of a chromene ring system in the molecule. The presence of two multiplets in the region 1.71 – 2.11 δ integrating to two methylene groups and the presence of two doublets at 5.51 δ and 6.67 δ and methyl groups revealed the presence of a geranyl side chain involved in the chromene ring formation [12]. This ring system was placed at C-3 and C-4 position of the pterocapan skeleton. Two *ortho* coupled doublets at 7.26 δ and 6.53 δ each integrating to one proton were assigned to H-1 and H-2 protons respectively.

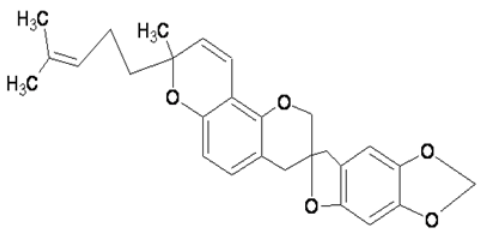

Fig. 1 Structure of Nitiducarpin

From the above NMR discussion, the structure of compound I was found to be identical with (+) nitiducarpin (I) earlier reported from other Dalbergia species [13].

The ^{13}C NMR spectrum of compound I (Fig. 1) exbihited signal corresponding to twenty six carbons in the molecule and all the signals were in accordance with the structure (I) proposed for compound I.

Based on the above spectral values the structure of compound I was inferred as nitiducarpin earlier reported from other Dalbergia species [13]. It was further confirmed by direct comparison with an authentic sample.

3.2 Investigation of the Alcohol Extract

The alcohol extract on concentration under reduced pressure gave a viscous brown pasty mass. It was found to be homogenous on TLC and hence subjected to column chromatographic separation. The column was built in pure chloroform and the elution was carried out by using solvents of increasing polarity. Elution of the column with pure chloroform yielded a small amount of nitiducarpin. Chloroform: ethanol (9.8:0.2) elutes give another compound designated as compound II. The structure of compound II was characterized as dalbergin by spectral studies.

3.2.1Compound II (Dalbergin)

The solid obtained from chloroform – ethanol eluate of the column, crystallized from methanol afforded a light brown crystalline solid. Compound II crystallized from methanol as light yellow colour solid, m.p. 216-218 °C. It was found to be homogenous on TLC using various solvents. It gave a negative test with alcoholic ferric chloride and sodium amalgam and concentrated hydrochloric acid indicating the absence of isoflavonoids. It did not give any colour with alcoholic ferric chloride solution indicating the absence of chelated hydroxyl group in the compound.

The UV spectrum of compound II in chloroform exhibited absorption maximum at 251 and 316 nm. The IR spectrum of compound II showed the presence of carbonyl group (1670 cm^{-1}) and hydroxyl group (3240 cm^{-1}). The compound formed mono acetate with acetic anhydride in pyridine solution indicating the presence of one hydroxyl group in compound (II).

The ^{1}H NMR spectrum of compound II revealed the presence of a methoxyl group, a hydroxyl, an unsubstituted phenyl ring and three protons singlets in the aromatic region. The NMR spectral values suggested that the compound II might be dalbergin. The three singlets at 6.26 δ, 6.90 δ and 6.99 δ each integrating to one proton was assigned to H-3, H-8 and H-5 protons respectively. The three protons singlet at 3.99 δ is due to methoxyl and five protons singlet at 7.48 δ is due to phenyl ring. Thus the structure of the compound was established as dalbergin (II).

The ^{13}C NMR spectrum of compound II (Fig. 2) exhibited signals corresponding to sixteen carbon atoms in the molecule and all the signals were in accordance with the structure proposed for compound (II). A comparison of the ^{1}H NMR and ^{13}C NMR spectrum of compound II with that of dalbergin reported in literature confirmed its identity.

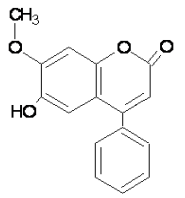

Fig. 2 Structure of dalbergin

3.2.2 Acetate of dalbergin

The acetate of dalbergin (II) was prepared by treating with acetic anhydride and dry pyridine at room temperature. The acetate has m.p. 148-150 °C. The melting points of the acetate derivative compared with the melting point of dalbergin acetate (lit. m.p. 156 °C).

4. Conclusion

The structure of two compounds viz., nitiducarpin and dalbergin, isolated from D*albergia rubiginosa* Stem. In case of dalbergin, the structure is confirmed by preparing the acetate, recording their spectra and comparing the values with the literature values.

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