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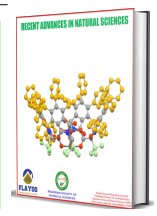

Isolation and characterization of a dihydrostilbene from the aerial parts of *Indigofera conferta*

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ABSTRACT

Indigofera conferta, an annual herb widely distributed in northeastern and western Nigeria, is used ethnomedicinally to treat inflammation, pain, sore feet, and snakebite. Despite these uses, the phytochemical constituents of this species remain underexplored. The chloroform fraction of the methanol extract of the aerial parts of *I. conferta* was separated by silica gel column chromatography to afford a compound. The structure of the compound was elucidated using UV, FT-IR, one-dimensional and two-dimensional NMR (^{1}H, ^{13}C, HSQC, and HMBC) spectroscopy and comparison with previously reported literature data. The compound was assigned as 3,5-dimethoxy-4′-O-(2,3-dihydroxy-3-methylbutyl)-dihydrostilbene. This report documents the first isolation of this compound from *I. conferta* and contributes to the phytochemical and chemotaxonomic knowledge of the genus *Indigofera*.

1. INTRODUCTION

Indigofera conferta Gillet is a member of the family Fabaceae. It is commonly found growing in red soil and is an annual herb with sticky, hairy leaves, branches, and flowers [1]. *Indigofera conferta* is widely distributed in northwestern Nigeria. Ethnomedicinal information from the Hausa people of northern Nigeria revealed that the decoction of leaves is used to treat sore feet [2]. It is also used in the management of inflammation, pain, and snakebite [3]. Despite its traditional medicinal importance, the phytochemical profile of *I. conferta* has not been extensively investigated. Previous phytochemical studies within the genus *Indigofera* have revealed diverse classes of secondary metabolites, including flavonoids, isoflavonoids [4], xanthenes [5], terpenoids, alkaloids, and phenylpropanoids [6, 7]. These metabolites are often considered valuable chemotaxonomic markers that contribute to understanding chemical diversity and evolutionary relationships within the genus.

In our earlier phytochemical investigation of *I. conferta*, we reported the isolation of a chalcone derivative, 2′,4′-dihydroxy-4-prenyloxychalcone [8]. This report provides additional evidence for the occurrence of phenolic constituents within this species. However, the constituents of this plant remain underexplored.

Dihydrostilbenes and related phenolic compounds have been reported from several species of the genus *Indigofera*. No-

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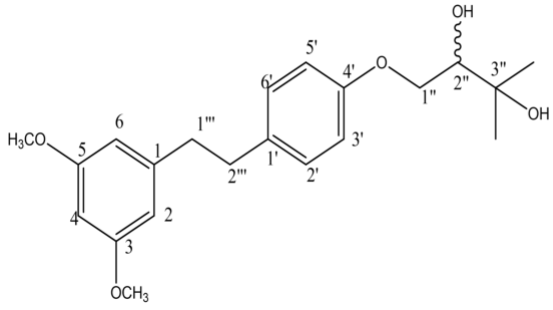

Figure 1. 3,5-Dimethoxy-4′-O-(2,3-dihydroxy-3-methylbutyl)-dihydrostilbene (R1).

tably, Musa *et al.* [9] reported the isolation of dihydrostilbene derivatives from *Indigofera pulchra*, including compounds bearing structural features closely related to the compound described herein. The occurrence of such dihydrostilbenes across multiple *Indigofera* species supports their value as chemotaxonomic markers for the genus. However, to the best of our knowledge, no report has previously described the isolation of a dihydrostilbene from *I. conferta*.

The present study was therefore undertaken to investigate the phytochemical constituents of the aerial parts of *I. conferta*. The isolation and structural characterization of a dihydrostilbene from this plant is the first report of such a compound from *I. conferta*. This finding extends comparative phytochemical knowledge and chemotaxonomic understanding within the genus *Indigofera* and may serve as a basis for future pharmacological investigations.

2. MATERIALS AND METHODS

2.1. COLLECTION, IDENTIFICATION, AND PREPARATION OF PLANT MATERIALS

The plant sample, comprising the leaves, stem bark, seeds, and fruits (aerial parts) of *Indigofera conferta*, was collected from Area BZ, Samaru-Zaria, Kaduna State, Nigeria, in October 2018. The plant sample was authenticated by Mr. Namadi Sanusi, a taxonomist at the herbarium section of the Department of Botany, Ahmadu Bello University, Zaria. A voucher specimen (No. 01084) was deposited at the herbarium. The aerial parts were shade-dried and pulverized manually using a mortar and pestle and are subsequently referred to as powdered plant material.

2.2. EXTRACTION

The powdered plant material (1400 g) was macerated in methanol (5 L), with occasional shaking, for 72 h and concentrated *in vacuo* to afford a deep green crude methanol extract (150 g), subsequently referred to as the methanol aerial extract (MAE). A portion of the crude extract (130 g) was suspended in distilled water and successively partitioned with *n*-hexane, chloroform, and ethyl acetate to yield the hexane fraction (HF), chloroform fraction (CF), and ethyl acetate fraction (EF), respectively.

2.3. CHROMATOGRAPHIC SEPARATION OF CHLOROFORM FRACTION

The chloroform fraction (7 g) was subjected to silica gel column chromatography (60–120 mesh) on a column (75 cm × 3.5 cm). The column was packed using the wet slurry method. The sample was adsorbed on silica gel by dissolving it in a minimal amount of chloroform, followed by the addition of silica gel to form a slurry. The mixture was dried and loaded onto the column. Elution was carried out using gradient mixtures of *n*-hexane and ethyl acetate, starting with 100% *n*-hexane (400 mL) and gradually increasing the polarity to *n*-hexane:ethyl acetate (35:65), followed by washing with 100% methanol. A total of 110 fractions (100 mL each) were collected and pooled based on TLC profiles to obtain fourteen combined fractions labelled A–N. Fraction M was further subjected to repeated column chromatography over silica gel to yield a white amorphous compound (8 mg) coded R1. Compound R1 (8 mg) corresponds to approximately 0.11% of the chloroform fraction (7 g) and approximately 0.00057% of the original dried plant material (1.4 kg). Such low yields are commonly observed in natural product isolation studies, as many secondary metabolites occur in plant matrices at trace levels [10]. The isolated compound was subjected to chemical and spectroscopic analysis to elucidate its chemical structure.

2.4. SPECTRAL ANALYSIS

UV spectra were recorded on a Shimadzu UV-2500PC spectrophotometer. IR spectra were recorded on a Shimadzu FTIR-8400S spectrophotometer. One-dimensional and two-dimensional ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE III spectrometer (600 MHz for ^{1}H and 150 MHz for ^{13}C) using the residual solvent peaks as internal standards. Chemical shift values (δ) are reported in parts per million (ppm) relative to the appropriate internal solvent standard, and coupling constants (J values) are given in hertz. Deuterated methanol was used as NMR solvent. High-resolution mass spectrometry (HRMS) data were not available for this study.

3. RESULTS AND DISCUSSION

The spectra and NMR data tables for compound R1 are provided in the Supplementary Material. Compound R1 was obtained as a white amorphous material and gave a positive reaction with ferric chloride reagent, indicating the presence of phenolic functional groups [11].

The UV spectrum (MeOH) showed absorption maxima at 362 and 289 nm, which are characteristic of conjugated aromatic systems typically found in stilbene derivatives. These absorptions are consistent with the presence of substituted phenyl rings within the molecular structure [12].

The IR spectrum showed a strong absorption band at 3279 cm^{-1} attributable to hydroxyl (O–H) stretching vibrations. The absorption band observed at 2985 cm^{-1} corresponds to aliphatic C–H stretching vibrations, while the band at 1604 cm^{-1} is characteristic of aromatic C=C stretching vibrations, indicating the presence of aromatic rings within the molecule. These spectral features are consistent with phenolic stilbene-type compounds previously reported in related species [13].

The ^{1}H NMR spectrum of compound R1 displayed signals for two methoxy groups (δ 3.70, 6H), as well as meta-coupled aro-

matic protons at δ 6.29 (2H, d, $J = 2.16$ Hz) and δ 6.27 (1H, d, $J = 2.16$ Hz). An AA′BB′ aromatic system was also observed at δ 7.05 (2H, d, $J = 8.58$ Hz) and δ 6.83 (2H, d, $J = 8.58$ Hz), suggesting the presence of a para-substituted benzene ring. Signals at δ 2.78 (2H, m) and δ 2.81 (2H, m) indicated the presence of two benzylic methylene groups typical of a dihydrostilbene skeleton. Additional resonances corresponding to two methyl groups at δ 1.22 and 1.25 (each 3H, s), a methine proton at δ 3.70 (1H), and oxymethylene protons at δ 3.88 and 4.19 further indicated the presence of a 2″,3″-dihydroxy-3″-methylbutyloxy side chain [14].

The ^{13}C NMR spectrum revealed twenty-one carbon signals, including methoxyl carbons, aromatic carbons, benzylic methylenes, oxygenated quaternary carbons, and carbons corresponding to the side chain. Sixteen carbons were assigned to the dihydrostilbene moiety, attributed to two methoxyl carbons, seven methine carbons, two benzylic methylene carbons, and five quaternary carbons. Five carbon signals were assigned to the O-prenylated units, attributed to two methyl signals, one methine carbon, one oxymethylene carbon, and one quaternary carbon.

The HSQC spectrum allowed direct correlation of proton signals with their respective carbons, while HMBC correlations established connectivity between different structural fragments. Key correlations included a ^3J correlation of δ_H 6.83 (H-3′ and H-5′) to a quaternary carbon at 135.4, confirming its assignment as C-1′. δ_H 7.05 (H-2′ and H-6′) exhibited a ^3J correlation to a benzylic methylene carbon at 38.1 (C-2‴) and to an oxygen-bearing aromatic quaternary carbon at 158.9 (C-4′). In the 2,3-dihydroxy-3-methylbutyloxy side chain, the methyl hydrogens showed a ^3J correlation to an oxymethine at 77.8 (C-2″) and to an aliphatic quaternary carbon at 72.9 (C-3″). The oxymethylene hydrogens (H-1″) at δ 3.88 (t, $J = 7.9, 9.9$ Hz) and 4.19 (d, $J = 2.8, 9.9$ Hz) exhibited a ^3J correlation to the oxygen-bearing quaternary carbon at 158.9 (C-4′), confirming attachment of the side chain at C-4′.

Although HRMS data were not available for this study, the proposed structure was assigned based on a comprehensive interpretation of UV, IR, one-dimensional and two-dimensional NMR (^{1}H, ^{13}C, HSQC, and HMBC) data and comparison with previously reported literature [9] (see Table S2, Supplementary Material). The NMR data of compound R1 showed close agreement with those reported for the corresponding dihydrostilbene from *Indigofera pulchra* by Musa *et al.* [9]. Accordingly, compound R1 was proposed to be 3,5-dimethoxy-4′-O-(2,3-dihydroxy-3-methylbutyl)-dihydrostilbene (Figure 1).

4. CONCLUSION

Phytochemical investigation using silica gel column chromatography led to the isolation of 3,5-dimethoxy-4′-O-(2,3-dihydroxy-3-methylbutyl)-dihydrostilbene from the methanol aerial parts extract of *Indigofera conferta*. This report documents the first isolation of this compound from *I. conferta*. The structural characterization of this dihydrostilbene provides additional evidence for the occurrence of phenolic constituents in *I. conferta* and contributes to the growing database of secondary metabolites reported for the genus *Indigofera*. The present work is primarily limited to phytochemical isolation and structural characterization; biological activity studies on the isolated compound are currently underway.

DATA AVAILABILITY

The data supporting the findings of this study are available in the attached Supplementary Material.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

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