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Isolation of 3, 5, 7-trihydroxy-2-(4-hydroxy-3-methoxyphenyl) chromen-4-one from the stem bark of *Lonchocarpus sericeus* Poir. (Papilionaceae)

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Abstract
An O-methylated flavonol 3, 5, 7-trihydroxy-2-(4-hydroxy-3-methoxyphenyl) chromen-4-one (isorhamnetin) was isolated from the dichloromethane fraction of the stem bark of *Lonchocarpus sericeus* Poir., (Papilionaceae), a medicinal plant widely employed in folkloric medicine in many communities in Southern Nigeria for the treatment of inflammation and pain. Its structure was established by a combination of spectroscopic techniques including FT-IR, ^{1}HNMR, ^{13}CNMR, 2-dimensional NMR spectroscopy, ESI-MS/MS and by direct comparison with literature values. This is the first report of the isolation of this compound from the plant *L. sericeus*.

Keywords: Ethnomedicine, chromatography, isorhamnetin, *Lonchocarpus sericeus*, spectroscopy

1. Introduction
Lonchocarpus sericeus Poir. (Papilionaceae) is a leguminous plant commonly known as Senegal lilac or Cube root. It is a dry deciduous tree that usually grows up to 16 meters high. It has dense hanging racemes of purple flowers which makes it perfect for display purposes. The flowers have a characteristic smell which is similar to vanilla [1-2]. In many parts of Southern Nigeria, leaves are used for general healing while the bark is useful for treatment of body pains, arthritis and rheumatism, cutaneous and subcutaneous parasitic infection. The roots are employed for treatment of leprosy. The fruit and seeds are commonly utilized as insect repellants [3].
There are scanty reports on previous works done on the stembark of the plant unlike other parts of the plant. The available reports include: isolation of a pentacyclic triterpenoid lupeol from the stembark of *L. sericeus* [4] as well as the anticonvulsant activity of methanol extract of the stem bark of *L. sericeus* [5]. Therefore, the present study is aimed at isolation and characterization of more bioactive compounds from the stembark of *L. sericeus*.

2. Materials and Methods
2.1 Plant materials
The fresh stembarks of *L. sericeus* were collected from a forest edge in Ikono Local Government Area of Akwa Ibom State, Nigeria and were identified and authenticated by a botanist Mr. Ndukwe Ibe of the Department of Forestry, Michael Okpara University of Agriculture, Umudike, Nigeria. The plant sample was further confirmed at the Pharmacognosy and Natural Medicine Department of the University of Uyo, Nigeria, where a voucher specimen (UUH 62/19) of the plant has been deposited in the Herbarium

2.2 Extraction and Partitioning
The plant part (stem barks) were washed and shade-dried for two weeks. The dried stembarks were further chopped into small pieces. The chopped stem barks (2.0 kg) was macerated in 97% methanol for 72 h to give the crude methanol extract. The liquid filtrate was concentrated and evaporated to dryness in *vacuo* at 40 ^{0} C using rotary evaporator. The dried crude extract was preserved in the refrigerator at 4 ^{0} C until further use.
The methanol extract of *L. sericeus* stem bark (100 g) was dispersed in 300 ml of distilled water and partitioned into dichloromethane (DCM) using a separating funnel.

The DCM fraction was subsequently concentrated under reduced pressure in a rotary evaporator (WG-EV311-V, Wilmad-LabGlass, USA) at 40 °C until they became completely dry. The DCM fraction (20.0 g) was put in a sealed glass sample container and kept in a refrigerator at 4 °C until analysis.

2.3 Isolation and purification of compound

The dichloromethane fraction (20.0 g) was submitted to silica gel column (600 g, 70-230 mesh, 600 mL) eluted with hexane/DCM/EtOAC gradient, yielding 126 fractions (D_1-D_{126}), which were pooled based on similarities in R_f values. Based on their TLC profiles, similar eluates were combined to yield 25 fractions. They were further combined to yield 13 fractions as follows: D_1-D_{31}, D_{32}-D_{49}, D_{50}-D_{69}, D_{70}-D_{75}, D_{76}-D_{80}, D_{81}-D_{86}, D_{87}-D_{94}, D_{95}-D_{102}, D_{103}-D_{110}, D_{111}-D_{116}, D_{117}-D_{120}, D_{121}-D_{125} and D_{126}. Fractions D_{50}-D_{69}, D_{70}-D_{75}, D_{76}-D_{80}, D_{81}-D_{86}, and D_{87}-D_{94} were further combined and subjected to column chromatography on silica gel (70-230 mesh; 600 mL), using a gradient of hexane/DCM/EtOAC to yield 16 fractions (E_1-E_{16}). Fractions E_1-E_7 was subjected to preparative TLC (hexane/EtOAC 3:1) and yielded a yellow solid named (LS) Repeated column chromatography of these sub-fractions did not yield any pure compound.

2.4 Spectroscopic characterization

The NMR of pure compounds was carried out on a Bruker AVANCE 400 (operating at 400 MHz for proton and 400 MHz for carbon). It was processed using a Bruker software. NMR spectra were calibrated using solvent signals (^{13}C: $CDCl_3$ δ 77.0 ppm; ^{1}H: $CHCl_3$ in $CDCl_3$ δ7.26 ppm). Chemical shifts were given in δ (ppm) and coupling constants reported in Hz. Structural assignments were based on the interpretation of the following NMR experiments: ^{1}H, ^{13}C, ^{1}H-^{13}C HSQC. Accurate mass was determined on Gas Chromatography/mass spectroscopy (GC/MS) performed on an Agilent 6890N/5973B GCMS system. Infrared spectra were recorded on a 5500 series compact FTIR (Agilent Technologies) instrument. Melting point was determined with a Gallenkamp melting point apparatus and it was not corrected.

Column chromatography was performed using silica gel (70-230 mesh, Sigma Aldrich) in glass columns of varying sizes fitted with Teflon taps. Analytical Thin Layer chromatography (TLC) was performed on pre-coated aluminium sheets with fluorescence (Silica gel $^{60}F_{253}$ 0.2mm thickness, Sigma Aldrich); preparative TLC was done with TLC plates with florescence (Silica gel $^{60}F_{253}$; 1mm thickness). Detection was done with iodine crystals or by visualization under ultraviolet light at wavelengths 254 and 366 nm.

3. Results and Discussion

3.1 Results

The compound (LS 3, 18 mg), appeared as a yellow amorphous solid with a melting point of 307 ^{0}C. IR ν_{max} (CCl_4) cm^{-1}: 3321, 2942, 2830, 1650, 1448, 1420, 1021;^{1}HNMR (400 MHz, $CDCl_3$): δ 12.22 (1H, s), 11.82 (1H, s), 7.26 (1H, s), 6.80 (1H, s), 6.51 (1H, m), 3.87 (3H, s); ^{13}CNMR (400 MHz, $CDCl_3$): δ 168.65 (C-4), 167.40 (C-7), 156.67 (C-5), 164.79 (C-9), 147.00 (C-2), 145.00 (C-4′), 142.00 (C-3′), 134.18 (C-3), 111.00 (C-6′), 111.00 (C-1′), 109.27 (C-5′), 108.03 (C-2′), 102.60 (C-10), 101.35 (C-6), 56.04 (3′-OMe), 29.88 (unassigned); ESI-MS/MS (negative mode) $C_{16}H_{12}O_7$ $[M-H]^-$ m/z 315, 300 (299), 287 (286), 271, 152 (151).

3.2 Discussion

The compound (LS 3, 18 mg), appeared as a yellow amorphous solid with a melting point of 307 ^{0}C. The spectrum of LS 3 showed a broad absorption around 3321 cm^{-1} indicative of a typical O-H bond vibration of a hydroxyl group. The absorptions at 2942 cm^{-1} and 2830 cm^{-1}are characteristic of the C-H stretching vibrations of a methyl moiety while its bending vibration was found around 1420 cm^{-1} as an absorption of medium intensity. The corresponding C=C vibration was shown around 1448 cm^{-1} as weakly intense band. The corresponding C-C vibration was shown as weak intense band at 1021 cm^{-1}. The weak absorption around 1650 cm^{-1} can be attributed to the C=O bond of a carbonyl compound.

The ESI-MS/MS determination (negative mode) yielded an m/z of 315 which represents $[M-H]^-$. This confirms a molecular mass of 316 corresponding to the formula $C_{16}H_{12}O_7$ with eleven degrees of unsaturation. These eleven elements of unsaturation are: seven C=C bonds, one C=O bond and three six-membered rings. Other prominent peaks were m/z 300 (299), m/z 287 (286), m/z 271 and m/z 152 (151). These peaks are in good agreement with 3, 5, 7-trihydroxy-2-(4-hydroxy-3-methoxyphenyl) chromen-4-one (isorhamnetin) [6].

In the ^{1}HNMR (400 MHz, $CDCl_3$) spectrum of LS 3, the chemical shift of the hydroxyl proton at carbon number five (C-5) on the A-ring appeared at 12.22 ppm (1H, s) while the signal at 11.82 ppm (1H, s) was assigned to the hydroxyl proton at carbon seven (C-7). The signal at 3.87 ppm (3H, s) was assigned to the three protons present in the 3′-OMe group on ring B. Other signals as shown in Table 4.15 were 7.26 ppm (1H, s) assigned to 3-OH proton, 6.80 ppm (1H, s) assigned to the 4′-OH proton and the multiplet at 6.51 ppm attributed to the proton at 6′-carbon position. These assignments are in good agreement with a similar report on preparative isolation of isorhamnetin from *Stigma maydis* using high-speed counter current chromatography [7].

The structural identity of LS 3 was further confirmed by its carbon NMR signals which showed signals for sixteen identifiable carbon atoms. The signal at 56.04 ppm was attributed to the –OCH_3 attachment to the B ring. The signal at 168.65 ppm is attributed to C-4. The signal at 156.67 ppm and 167.40 ppm can be attributed to C-5 and C-7 respectively. Other chemical shift values shown in Table 4.15 are very consistent for 3, 5, 7-trihydroxy-2-(4-hydroxy-3-methoxyphenyl) chromen-4-one (isorhamnetin) when compared with values in literature [7].

The signal at 29.88 ppm is due to NMR artifact peak and not an experimental value. Improper setting of number of scans, repetition time and pulse duration among other reasons have been adduced to explain the presence of unwanted artifacts and inaccurate spectral properties in an NMR experiment [8]. The ^{1}H-^{1}H Correlation Spectroscopy (CosY) and Heteronuclear Multiple Quantum Coherence (HMQC) data also supported the earlier mentioned assignments as characteristic of isorhamnetin.

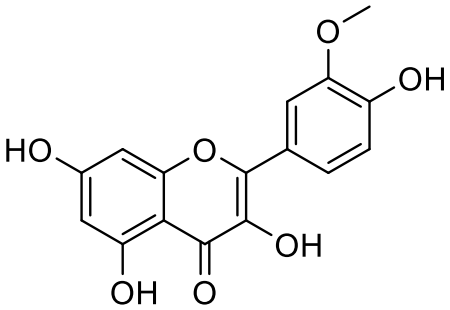

Fig 1: LS 3: 3, 5, 7-trihydroxy-2-(4-hydroxy-3-methoxyphenyl) chromen-4-one

4. Conclusion

The dichloromethane fraction of the stem bark of *Lonchocarpus sericeus* chromatographed on silica gel in hexane/DCM/EtOAC afforded a yellow solid substance code named LS 3. Physical and spectral characteristics of LS 3 as well as comparison with literature data have confirmed its identity as 3, 5, 7-trihydroxy-2-(4-hydroxy-3-methoxyphenyl) chromen-4-one, an O-methylated flavonol.

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6. Conflicts of interest: Authors declared there are no conflicts of interest to disclose.

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