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CAFFEOYL GLYCOSIDE AND A LIGNAN FROM THE METHANOL STEM BARK EXTRACT OF *LONCHOCARPUS SERICEUS* POIR (FABACEAE)

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ABSTRACT

Lonchocarpus sericeus Poir (Fabaceae) is a leguminous plant belonging to the family Fabaceae widely distributed throughout tropical and subtropical regions of Africa, particularly in Nigeria, Niger, Togo, Benin, Ghana, and Chad. It is utilized in ethnomedicine to treat microbial infections, back pain, convulsions, leprosy, and inflammation among others. Caffeoyl glycoside and Acantrifoside F were isolated from the ethylacetate soluble fraction of the methanol extract of *Lonchocarpus sericeus* stem bark using a combination of silica gel column chromatography and gel filtration, the structures of the compounds were elucidated with the aid of IR, 1D-^{1}H and ^{13}C NMR spectroscopic analysis.

Keywords: Acantrifoside F, Caffeoyl glycoside, *Lonchocarpus sericeus*, NMR analysis, stem bark,
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INTRODUCTION

Plants are a key component of traditional medicine because they contain bioactive chemical compounds (secondary metabolites) that have a clear physiological effect on the body [1]. Secondary metabolites from plants are valuable for the economy, used in pharmaceuticals, perfumes, insecticides and dyes, they have distinct chemical structures and are rapidly discovered, despite their classification [2].

Lonchocarpus sericeus is a leguminous plant which is commonly called Senegal lilac or Cube root in English, *Furan yarsarki* or *Shunin biri* in Hausa, while in Igbo it is known as *Njasi* and popularly called *Apapo* in Yoruba.It is commonly planted in villages as a shade tree and in gardens [3, 4]. In Nigeria, leaves are employed for holistic healing while the bark is exploited for the management of body pains, arthritis, rheumatism, cutaneous and subcutaneous parasitic infection, malnutrition, debility, paralysis, and convulsions [5].

Previous phytochemical screening of the methanol extract of the stem bark of *Lonchocarpus sericeus* showed the presence of alkaloids, flavonoids, tannins, saponins, and steroids [6]. Prenylated Chalcone and Lupeol were previously reported from the chloroform soluble fraction [7], while beta-stigmasterol and a chromen-4-one were isolated from the hexane and dichloromethane fraction [8, 9]. In this work, we report herein the isolation of a caffeoyl glycoside and a lignan
from the ethylacetate fraction of the methanol extract of the stem bark of *Lonchocarpus sericeus* using chromatographic techniques.

MATERIALS AND METHODS

Collection and identification of the plant material

The stem bark of *Lonchocarpus sericeus* was collected from Kongo campus of Ahmadu Bello University Zaria after identification of the tree on the field using descriptions in the monograph [10]. The leaves of the plant were also collected alongside to ease identification of the bark. The identity of the bark was confirmed and authenticated by taxonomist Namadi Sanusi at the Herbarium unit, department of Botany, Ahmadu Bello University Zaria by comparison with existing herbarium specimen of voucher number [V/NO:1085].

Preparation and fractionation of the crude extract

The stem bark was dried at room temperature for several weeks and size reduced manually using mortar and pestle. The size reduced stem bark (1.5 kg) was extracted using cold maceration with methanol for four days with occasional shaking and swirling, the extract was concentrated *in vacuo* using rotary evaporator at 40ºC to afford a greenish-brown crude methanol extract (CME).

This yielded 176 g of a gummy extract and was suspended in distilled water and filtered. The insoluble and soluble portion were separately partitioned with n-hexane, chloroform, and ethyl acetate in order of increasing polarity to obtain hexane fraction (HF), chloroform fraction (CF), and ethyl acetate fraction (EAF) respectively, these fractions were subjected to thin layer chromatography and ethylacetate soluble fraction was subjected to further phytochemical investigation because of it promising spots on thin layer chromatography.

Isolation and purification of compound LS1 and compound LS2

The ethyl acetate fraction (15 g) was subjected to column chromatography on a silica gel (60-120 mesh) with gradient elution using chloroform, ethylacetate and methanol respectively. Eluents were collected in 50 ml aliquot and TLC was used to monitor the progress of elution, a total of 250 fractions were collected and pooled together into 19 bulk major fractions (A-R) based on their TLC profiles. Column fraction K (10 mg) which showed three (3) spots was subjected to purification using silica gel(60-120 mesh) with gradient elution using ethylacetate and methanol, eluents were collected in 25 ml aliquots and progress of elution was monitored with TLC, a total of 200 fractions were collected and pooled together in 10 major sub-fractions (A-J) based on their TLC profiles, sub-fraction B showed two spots was subjected to repeated gel filtration using Sephadex LH_{20} and eluted with absolute methanol to give 5.0 mg yellow amorphous compound coded LS1. Compound LS1 gave a single homogenous spot with a solvent system of ethylacetate: chloroform: methanol: water (E: C: M: W) 15:8:4:1 and ethylacetate: methanol (E:M) 9:1.

Column fraction N from the pooled 19 bulk major fractions (A-R) which showed two (2) spots was subjected to repeated gel filtration using Sephadex LH_{20} and eluted with absolute methanol to give 5.0 mg light brown amorphous compound coded LS2. Compound LS2 gave a single homogenous spot with solvent system ethyl acetate: methanol 4:0.5. The isolated compounds were subjected to physicochemical tests as well as spectroscopic analysis to elucidate their chemical structures.

RESULTS AND DISCUSSION

Compound LS1 was isolated as a yellowish amorphous compound with a melting point of 275-277°C, with R_f value 0.47 and gave a positive ferric chloride test indicating the presence of a phenolic nucleus [11]. The IR spectrum of compound LS1 revealed absorption frequency at 3336 cm^{-1} typical of O-H stretching, 2929.7 $cm^{-1,}$ due to CH_2 asymmetric stretch, other bands observed at 1692 cm^{-1} were due to C=O stretching in conjugates esters, 1599 cm^{-1} and 1515 cm^{-1} were due to the aromatic ring, and 1602 cm^{-1} indicating C=C [12].

The ^{1}HNMR spectrum of compound LS1 revealed signals of an ABX system of two trisubstituted rings, ring 1 signal at δ_H7.03 (1H,s, H-2), δ_H6.77 (1H,d,J=8Hz, H-5), δ_H6.89 (1H,d,J=8Hz,H-6),and signal at δ_H7.35 (1H,s H-2'), δ_H6.64 (2H,m, H-5'), δ_H6.52 (2H,m, H -6') for ring 2. Two trans vinylic protons signals at δ_H 7.54 (1H, d,J= 16Hz) for H-7, δ_H 6.29 (1H, d,J=16Hz) for H-8 and δ_H 7.52 (1H, d,J= 12Hz) for H-7', 6.29 (1H, d, J=12) for H-8', two anomeric proton signals at δ_H5.17and 4.49 (1H, brs), overlapping carbinol proton signals at δH 3.30-4.49 (10H) typical characteristic signals of two (trans)-caffeoyl units and two glucose units in close agreement with values reported for caffeoyl glycoside [12], it also shows the presence of terminal methyl proton resonance at δH 1.08 (3H,s).

The ^{13}C NMR spectrum of compound LS1 revealed the presence of thirty carbon resonances of which Δc 114.9, Δc 115.6, Δc 121.7 for C-2, C-5, C-6, for aromatic carbon in ring 2 and Δc 115.1, Δc 115.6, Δc 119.8 for C-2', C-5', C-6' for aromatic carbon in ring 1. Δc 145.8 C-3, Δc 148.2 C-4, Δc 146.4, C-3'and Δc 146.2, C-4' for quaternary aromatic carbons, chemical shift signals of Δc 146.6, C-7, Δc 113.6, C-8, Δc 114.6, C-7'and Δc 115.1, C-8' for the olefinic carbons, Δc 166.6, C-9 and Δc 167.5, C-9' for two carbonyl carbons, also presence of two anomeric carbons at Δc 129.9, C-1' and Δc 101.2, C-1''. Based on the above spectral analysis, all the data were in agreement with that of a caffeoyl glycoside [12, 13]. Compound LS1 was confirmed to be *E*-caffeoyl-6-O-[4-O-(*β-D*-rhamnosyl) *E*-caffeoyl]-*β-D*-glucopyranoside (Figure 1).

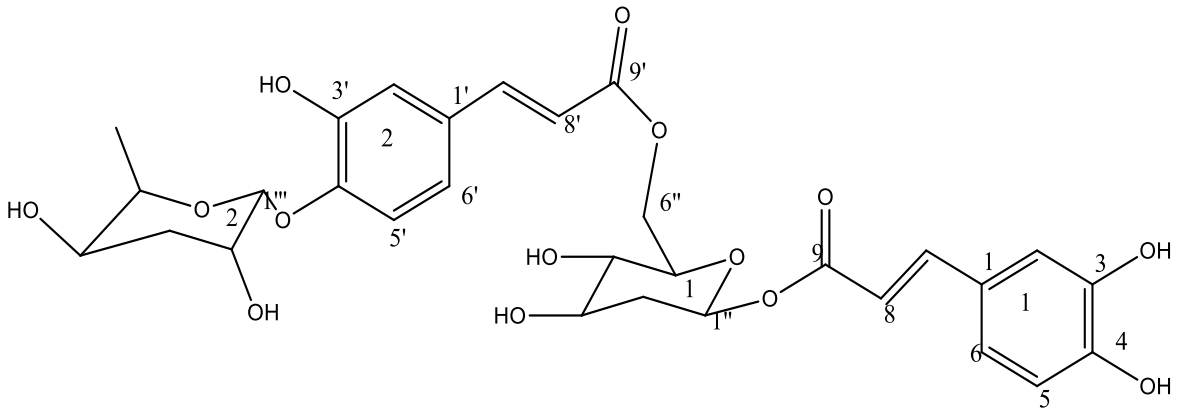

Figure 1: *E*-caffeoyl-6-O-[4-O-(*β-D*-rhamnosyl) *E*-caffeoyl]-*β-D*-glucopyranoside

Compound LS2 was isolated as white amorphous powder with a melting point of 318-320^{O}C. It showed a brown spot of R_f value 0.39 and showed positive to ferric chloride test indicating the presence of phenolic nucleus [11]. The IR spectrum of compound LS2 revealed broad band at 3339.7 cm^{-1} typical of O-H stretching frequency of a hydroxyl functional group, band at 2493.6 and 2855.1 cm^{-1} typical of symmetric and asymmetric C-H stretching of an aliphatic group, the 1036.2 cm^{-1} is typical of C-O-C stretching.

The ^{1}H-NMR spectrum of compound LS2 showed signals due to a methyl group at δH 1.86 (3H, d, J = 6.4 Hz), two methoxy proton resonance at δH 3.84 (6H, s), two olefinic protons resonance at δH 6.20 (1H, d, J= 16 Hz) and δH 6.33 (1H,d, J = 16 Hz) in trans-configurations, two protons resonance of an aromatic ring at δH 6.72 (2H, s, H-3, 5), two protons resonance for hydroxyl group at δH 3.83 (1H, dd, J = 12.0, 5.4 Hz) and δH 3.65 (1H, dd, J = 12.0 Hz), and anomeric proton resonance at δH 4.01. The ^{13}C-NMR spectrum revealed 21 carbon signals, including a methyl group at δ 18.4, methoxy groups at δ 64.8, anomeric carbon at δ 110.1, presence of 12 sugar signals at δ (103.0- 64.8) indicate two sugar moieties. Based on above data and comparison with the reported data [14,15]. Compound LS2 was confirmed to be 1-[*β-D*-glucopyranosyl-(1→6)-*β-D*-glucopyranosyl] - 2, 6-dimethoxy-4-propenylphenol (Figure 2).

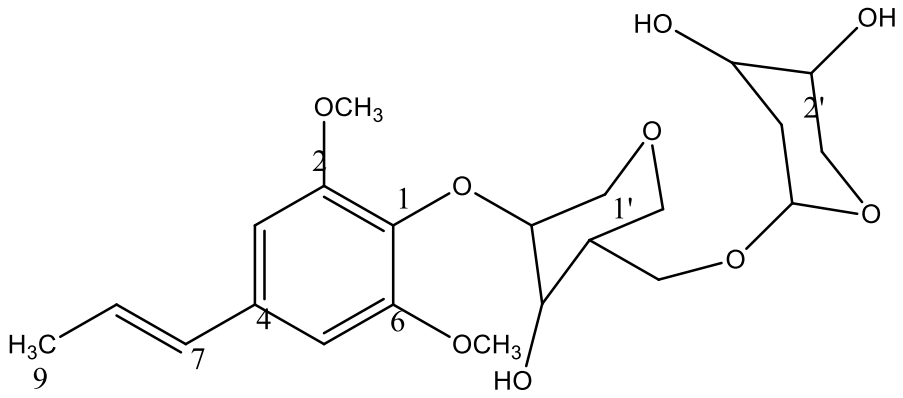

Figure 2: 1-[*β-D*-glucopyranosyl-(1→6)-*β-D*-glucopyranosyl]- 2,6-dimethoxy-4-propenylphenol (Acantrifoside F)

Table 1: Comparison of ^{1}H-NMR and ^{13}C-NMR of compound LS1 with that of caffeoyl glycoside data reported in the literature

Data from literature (Zhu *et al.*, 2008)			**Compound LS1**	
Carbon	δH (mult, J Hz)	δc	δH (mult, J Hz)	δc
1		125.3		1266.2
2	7.06(brs)	114.9	7.03(s)	114.9
3		145.6		145.8
4		148.7		148.2
5	6.77 (d, 8 Hz)	115.8	6.77 (d, 8 Hz)	115.6
6	7.02 (d,8 Hz)	121.7	6.89 (d, 8 Hz)	121.7
7	7.57 (d, 16 Hz)	146.6	7.54 (d,16 Hz)	146.6
8	6.26 (d, 16 Hz)	113.2	6.29 (d, 16 Hz)	113.6
9		165.2		166.6
1^{1}		128.6		129.9
2^{1}		115.1		115.1
3^{1}		146.8		146.4
4^{1}		147.5		146.2
5^{1}	7.12 (m)	116.1	6.64 (m)	115.6
6^{1}	7.12 (m)	120.9	6.52 (m)	119.8
7^{1}	7.52 (d, 16 Hz)	144.8	7.52(d, 12 Hz)	144.6
8^{1}	6.54 (d, 16 Hz)	115.8	6.29 (d, 12 Hz)	115.1
9^{1}		167.5		167.5
1^{11}	5.50 (d, 8 Hz)	94.1	5.17 (s)	101.2

2^{11}	3.20-3.35	72.4	3.30-3.39	72.5
3^{11}	3.20-3.38	76.1	3.30-3.39	74.2
4^{11}	3.58 (s)	69.6	3.30-3.39	68.9
5^{11}	3.38-3.58	74.6	3.30-3.39	73.9
6^{11}	4.41 (d, 11 Hz)	63.4	4.49 (d, 12 Hz)	63.1
1^{111}	4.18 (d,8 Hz)	101.6	4.46 (d, 8 Hz)	102.9
2^{111}	3.20-3.38	75.6	3.20-3.38	71.01
3^{111}	3.20-3.38	73.2	3.20-3.38	70.9
4^{111}		69.8		68.9
5^{111}	3.20-3.38	77.2	3.20-3.38	70.8
6^{111}	3.71	60.7	1.08 (s)	16.8

Table 2: Comparison of ^{1}H-NMR and ^{13}C-NMR of compound LS2 with that of Acantrifoside F data reported in the literature

	Data from literature (Kiem *et al*., 2013)		**Compound LS2**	
Carbon	**δH**	**δc**	**δH**	**δc**
1		132.9		132.8
2		152.7		145.9
3/5	6.65(br s)	103.5	6.72(d)	100.7
4		132.7		130.3
6		152.7		145.9
7	6.32(d)	130.2	6.33(d)	128.6
8	6.21(d)	124.3	6.20(d)	124.3
9	1.08	17.5	1.08	18.4
10	3.75	57.8	3.79	61.4
1^{1}	4.87(d)	102	4.45(d)	110.1
2^{1}	3.20	75.9	3.20	75.9
3^{1}	3.20	78.6	3.20	76.6
4^{1}	3.43	71.7	3.47	72.4
5^{1}	3.20	75.7	3.20	74.8
6^{1}	3.53	67.5	3.83	67.8
1^{1}	4.50	103.0	4.01	96.6
2^{1}	3.40	73.4	3.65	73.4
3^{1}	3.40	75.8	3.65	76.6
4^{1}	3.40	68.9	3.03	69.8
5^{1}	3.40	75.5	3.03	75.5
6^{1}	3.60	64.8	3.65	64.8

CONCLUSION

Caffeoyl glycoside and acantrifoside were isolated from ethylacetate soluble fraction of the stem bark extract of *Lonchocarpus sericeus*. The structures of these compounds were identified as *E*-caffeoyl-6-O-[4-O-(*β-D*-rhamnosyl)*E*-caffeoyl]-*β-D*-glucopyranoside (LS1) and 1-[*β-D*-glucopyranosyl-(1→6)-*β-D*-glucopyranosyl] - 2, 6-dimethoxy-4-propenylphenol (LS2) on the basis of their spectroscopic, comparing their physical properties and spectral data to those reported in the literature respectively.

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