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Isoliquiritigenin and β-sitosterol from *Cissus polyantha* Tuber Glig and Brandt

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Chemical investigation into the composition of *Cissus polyantha* tuber, a plant used in Northern Nigerian ethno medicine for managing inflammatory conditions and conjunctivitis has led to the isolation of a chalcone from the ethyl acetate and a steroid from the hexane soluble fractions of its methanol extract. The structures of these compounds were elucidated by extensive analysis of their spectroscopic data, including 1 and 2D nuclear magnetic resonance (NMR). This is the first report of the isolation of the compounds from the plant and they may be responsible for the previously reported anti-inflammatory effects.

Key words: *Cissus polyantha,* vitaceae, 2D nuclear magnetic resonance (NMR), chalcone, steroid.

INTRODUCTION

Medicinal plants are known to provide a rich source of raw materials used in traditional medicine practice in Africa and other parts of the developing world. Also, research and development from traditional medicinal preparations have led to the discovery of many potent drugs which are used in modern clinical practice (Burkill, 2000). Previous studies have shown that 61% of some 877 drugs introduced worldwide can be traced or were inspired by natural products (Cseke et al., 2004). *Cissus polyantha* is a semi-woody climber; found usually in closed-forest from Sierra Leone to Southern Nigeria and also from Eastern Cameroun to Ubangi (Burkill, 2000). *C. polyantha* has been used in the management of inflammatory conditions and diseases related to bacterial infections (Burkill, 2000). Previous biological studies on the plant have established its analgesic, anti-inflammatory and antimicrobial activities (Sani et al., 2013). In furtherance of our phytochemical investigations into *C. polyantha*, this paper reports the isolation of a chalcone from the ethyl acetate and a steroid from the hexane soluble fractions of the methanol extract of the tuber of the plant.

MATERIALS AND METHODS

General procedures

Nuclear magnetic resonance (NMR)-spectra were recorded on a

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Bruker AVANCE spectrometer (400 MHz) for ^{1}H- and ^{13}C-NMR (100 MHz). Residual solvent signal with chloroform was used as the internal standard and chloroform was used as the solvents. Melting points were determined on a Yanaco MP-400 micro melting point apparatus. For thin layer chromatography (TLC) analysis, silica gel 60 F_{254} (Merck) was used, column chromatography was performed using Merck silica gel (60 to 120) mesh, while gel filtration chromatography was performed using Sephadex LH-20 (Sigma, Spruce street, St. Louis, USA). Spots on TLC plates were visualized by spraying with 10% H_2SO_4 followed by heating at 100°C for 2 min.

Plant

Whole plant of *C. polyantha* growing in the wild was collected from Turunku village in Igabi Local Government Area, Kaduna State, Nigeria, in the month of June, 2009. It was authenticated by Malam U. S. Galla of the herbarium unit of the Biological Sciences Department, Ahmadu Bello University, Zaria, Nigeria, by comparing with an existing specimen (voucher No. 616). The tuber was separated from the plant, washed, sliced, air-dried and ground into powder using pestle and mortar, and subsequently referred to as powdered plant material.

Extraction and isolation

The powdered plant (1000 g) was extracted with 75% methanol in a Soxhlet apparatus for 48 h, the solvent was removed *in-vacuo* to yield a residue (80 g) referred to as crude methanol extract of the tuber of *C. polyantha* (CMET). The methanol extract (70 g) was suspended in water and partitioned successively with hexane, chloroform and ethyl acetate to obtain the hexane and ethyl acetate soluble fraction used for the study. The ethyl acetate fraction (7.0 g) was chromatographed on silica gel (60-120 mesh), and the packed column was eluted using gradient solvent systems of n-Hexane 100%, hexane/chloroform mixtures, chloroform 100%, and chloroform/ethyl acetate mixtures. 42 fractions of 100 ml each were collected. The fractions were pooled together based on their TLC profile to give 5 sub fractions. Repeated Sephadex LH-20 (MeOH) gel filtration chromatography of sub fraction 3 led to the isolation of compound 1 (6.2 mg). Similarly, the hexane fraction (7.0 g) was chromatographed on a silica gel column eluting with 100% hexane, hexane/chloroform mixtures and 100% chloroform as solvent systems to give 37 fractions. The 37 fractions were pooled together based on similarities in their TLC profiles to give 5 sub fractions. Preparative TLC of sub fraction 3 led to the isolation of compound 2 (7.4 mg).

RESULTS AND DISCUSSION

Compound 1 was isolated as a yellow solid with a melting point of 202 to 204°C; it gave a positive result to the Shinoda test for flavonoids. The ^{1}H NMR spectrum of compound 1 revealed signals for a pair of ortho coupled protons at δ_H 7.9 (d, 1H, *J* = 8.94 Hz, H-5'), δ_H 6.43 (dd, 1H, *J* = 8.9, 2.2 Hz, H-6') and a third signal at δ_H 6.25 (d, 1H, *J*= 2.2 Hz, H-3') which was a meta coupled to δ_H 6.43 (dd, 1H, *J* = 8.9, 2.2 Hz), assignable to the tri-substituted benzene ring A. The signals at δ_H 7.63 (d, 2H, *J* = 8.4 Hz, H-2, 6) and δ_H 6.85 (d, 2H, *J* = 8.4 Hz, H-3, 5) were assigned to the 1,4 di substituted ring B. Two trans-coupled olefinic proton at δ_H7.8 (d, 1H, *J* = 15.4 Hz, H-α) and δ_H7.6 (d, 1H, *J* = 15 Hz, H-β) were also assigned. These proton NMR signal assignments were supported by H^1-H^1 COSY correlations of H-5'/H-6', H-2/H-3 and H-α/H-β. The ^{13}C spectrum of compound 1 revealed a total of 12 signals, which were assigned to a ketone carbonyl at δ_C 193.30, the remaining signal were all for unsaturated carbon atoms at δ_C 102.55, 115.54, 116.99, 119.30 126.45, 130.39, 131.93, 144.08, 160.00, 160.20 and 165.00. They were assigned to two benzene rings and a two carbon atoms unsaturated olefinic system. The downfield signals at δ_C 144.08, 160.00 and 165.00 suggested that they are oxygenated carbon atoms. These ^{1}H and ^{13}C signals were very similar to those of isoliquiritigenin and a comparison of these data with those reported in the literature for isoliquiritigenin showed very close agreement (Markham and Ternai, 1976; Aida et al., 1990; Sato et al., 2007), hence the structure of compound 1 was determined to be isoliquiritigenin (Figure 1).

Compound 2 was isolated as a white amorphous solid with a melting point of 137 to 139°C; it gave a positive result to the Salkowski's test for steroid/triterpenes. Its ^{1}H-NMR spectrum revealed three regions typical of the steroidal nucleus at 0.5 to 2.5 ppm representing overlapping methyl, methylene and methine protons; an oxymethine proton at 3.5 ppm was assigned to the position 3 of the steroidal nucleus and a single unsaturated proton signal at 5.8 ppm. The ^{13}C NMR spectrum of compound 2 revealed a total of 29 carbon signals, 6 of which were methyl signals, 11 were methylene carbon signals, 9 were methine and there were 3 quaternary signals, these data is typical of β-sitosterol (Rowshanul et al., 2007; Pateh et al., 2009; Li et al., 2009; Hamada et al., 2012). The signal between 11.8 and 56.7 ppm represents a region of overlapping methyl, methylene and methine carbon atoms, an oxymethine signal at 71.8 was typical of position 3 of β-sitosterol, and finally the unsaturated carbon signals at 121.7 and 140.7 ppm were assigned to a two carbon olefinic system. These NMR data are very similar to the data for β-Sitosterol and a comparison with data reported for β-sitosterol showed good agreement (Rowshanul et al., 2007; Pateh et al., 2009; Li et al., 2009; Hamada et al., 2012). Also, Co-TLC analysis of compound 2 with standard sample of β-sitosterol showed them to have the same R_f value. Therefore, the structure of compound 2 was determined to be β-sitosterol (Figure 2).

A lot of studies on the biological activities of isoliquiritigenin and β-sitosterol have been reported in the literature; isoliquiritigenin has been reported to have several biological activities, including anti inflammatory, anti oxidant, anti tumor and anti spasmodic activities (Sato et al., 2007; Li et al., 2009; Hamada et al., 2012; Kim et al., 2008). β-sitosterol in addition to its well known

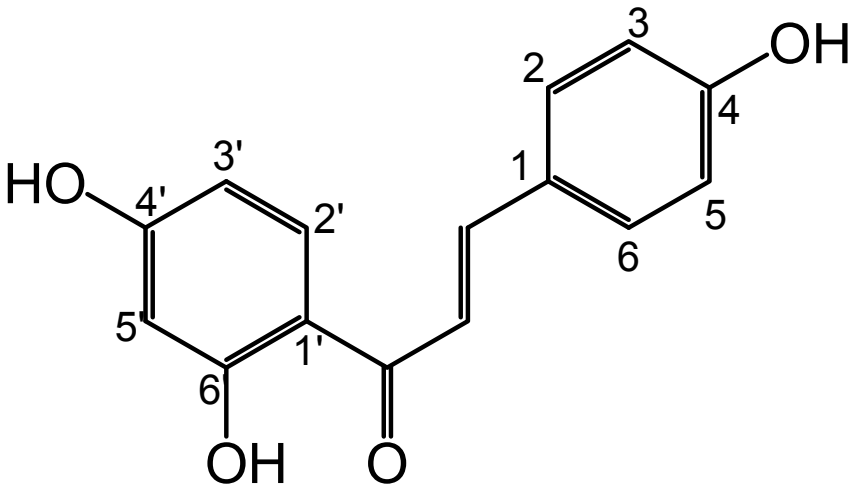

Figure 1. Isoliquiritigenin.

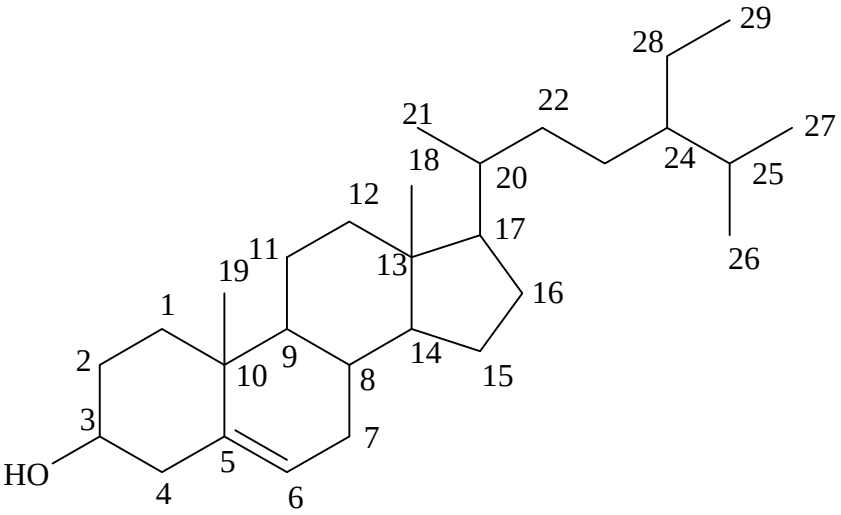

Figure 2. β-sitosterol.

anti-inflammatory effect has been reported to have analgesic, anti-oxidant, anti-diabetic, anthelminthic and anti-mutagenic activities (Saeidnia et al., 2014). These two compounds might therefore be partly responsible for the observed biological activities previously reported for *C. polyantha*, and their presence justifies the ethno medicinal claims on the plant.

Conclusion

The isolation of these two compounds, well known for their anti inflammatory and analgesic activities lends full justification to the ethno medicinal use of the plant in the treatment of inflammatory conditions.

Conflict of interest

The authors declare that they have no conflict of interest.

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