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Isolation and Characterization of the Chemical Structure of Compound in Methanol Extract of Cochlospermum planchonii Hook.F (Cochlospermaceae)

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Abstract
Cochlospermum planchonii Hook.F (Cochlospermaceae) leaf is used by the Hausa/Fulani in the Northern Nigeria for the management of pain, inflammation and related diseases yet, its chemistry is poorly known. The present study is to isolate and characterize the structure (s) of compound (s) in methanol extract of *Cochlospermum planchonii*. Fractions of methanol samples were fractionated using TLC method into 80 fractions. Based on TLC-DPPH assay, similar fractions were pooled together by Preparative TLC. Aromatic singlet proton at δ 8.03 in the 'H NMR spectrum indicates that the compound might be an isoflavone. The final structure of the compound was confirmed by comparing the NMR data of the compound with the literature data. The NMR spectrum was obtained in methanol- δ_4 and the literature values were obtained in DMSO.
Keywords: *Cochlospermum planchonii*, Isolation, Characterization, Singlet proton

1. Introduction

The advances in methodologies for separation technologies such as high Performance Liquid Chromatography (HPLC) and countercurrent partition chromatography have further expanded the capacity for separations of plants extracts (Pauli, 2006). Structure elucidation technology has improved especially with the development of high field Nuclear Magnetic Resonance (NMR) (Korfmacher, 2005; Deng and Sanyal, 2006; Phillipson 2007). This allows rapid and straightforward structure elucidation. Although the active principle isolated from plants may not necessarily be replaced by the plant extracts (Phillipson, 2001), drugs are now discovered as a result of chemical studies directed at the isolation of the active substances from plants used in traditional medicine where the drugs do not possess the optimal properties for their use in human or animal medicine, they would be subjected to structure modifications in order to improve their biological properties (Guthikonda *et al*., 1987).

Tremendous progresses have been made in the study of natural products during the last few decades. Advances in Nuclear Magnetic Resonance (NMR) spectroscopy have revolutionized the entire scenario of drug discovery. Structure elucidation of natural products which used to be bottleneck can now be carried out overnight using modern high field NMR instruments (Noda, 2008). With the development of computer assisted structure elucidation (CASE) programmer, it is now possible to generate all possible structures that are consistent with a particular set of spectroscopic data with minimum amount of human intervention (Jaspars, 1999; Blinov *et al*., 2003). Proton Nuclear Magnetic Resonance (^{1}HNMR) can provide adequate information such as number of protons present in the molecule, chemical and electronic environment of the protons, and the number of neighboring (vicinal or geminal) protons. It is also possible to distinguish among various types of carbons such as the primary (1^o), secondary (2^o), tertiary (3^o) and quaternary (4^o) from the carbon-13 (^{13}CNMR) spectra with the experiments such as *D*istortionless *E*nhanced *P*olarization *T*ransfer (DEPT).

The two dimensional NMR experiments (2D-NMR) show correlation and connectivity of the different groups within the molecule (Meusinger, 2006). Correlation spectroscopy (^{1}H COSY) shows coupling among neighboring protons with two or three bonds apart. *H*eteronuclear *M*ultiple *Q*uantum *C*orrelation (HMQC) shows proton and the carbon to which is directly attached i.e. one-bond (^{2}*J*) coupling (Bross-Walch *et al*., 2005). Since quaternary carbons have no attached protons, they don't appear in HMQC plots. The *H*eteronuclear *M*ultiple *B*ond *C*orrelation (HMBC) experiment detects long range coupling between proton and carbon (two or three bonds away) with great sensitivity. The correlation between carbons is obtained using the *I*ncredible-*N*atural *A*bundance-*D*ouble-*Q*uantum-*T*ransfer spectroscopy (INADEQUATE). It is a specially designed COSY experiment that yields ^{13}C-^{13}C correlations efficiently suppressing the much more abundant ^{13}C-^{12}C signals. To determine the relative stereochemistry of the molecule, ^{1}H coupling constant (*J*) and *N*uclear *O*verhauser *En*hancement *S*pectroscopy (NOESY) data is used (Neri & Tringali, 2004).

Although NMR is the most useful source of information for structure elucidation, it also needs to be complemented by other methods especially mass spectroscopy in order to obtain the molecular mass of the compound. Other techniques include the infrared (IR) spectroscopy which reveals information relating to the

functional groups, and the ultraviolet (UV) spectroscopy which reveals information relating to the presence of sites of un-saturation in the structure. These two methods are becoming less important in structure elucidation of natural products due to the superiority of information obtained from the NMR experiments (Houghton, 2005).

The Chemistry of Cochlospermaceae is poorly known especially, *Cochlospermum planchonii* (Paulo & Houghton, 2013) yet the Hausa/Fulani in the Northern Nigeria used this plant in the management of pain, inflammation and related diseases. Therefore, the present study is to isolate and characterize the structure (s) of compound (s) in methanol leaf extract of *Cochlospermum planchonii*.

2. Materials and Methods

2.1 Plant Collection, Identification and Preparation

Cochlospermum planchonii leaf was collected on the 9th June, 2012 from 'Babare' locality, in Jos North Local Government Area of Plateau state, Nigeria. The plant was identified in the field using the pharmacognostic descriptions and keys in official books. The identity of the plant was authenticated at the Department of Horticulture and Landscape Technology, Federal College of Forestry, Jos, Nigeria, and assigned Voucher specimen Number (FHJ 1010). The plant was collected and air dried at room temperature under shade until a constant weight was obtained for a period of three weeks. The plant was then pounded to powder using local pestle and mortar, sieved with a mesh of size-20 and stored in an air-tight container until when required for use.

2.2 Chemicals and reagents

All the solvents used in the study were of Analytical grade.

2.3 Plant Extraction

The powder drug (1kg) was extracted with methanol (3L) by maceration/electric shaking for 72 h. The extract was concentrated under reduced pressure using Rota-vapor to have methanol extract (18.40 % yield) using the standard method described by (Paschal *et al.,* 2002) with slight modifications.

2.4 Silica-gel Column Chromatography

Silica gel (l00 g, 40-63 µm) was carefully parked using wet method to about 30 cm high in a heavy walled glass tube leaving 60 cm headspace. The extract (3 g) pre-adsorbed on silica was loaded onto the parked adsorbent and allowed to stabilized for 3 hours before elution begins. Hexane (100 %) was used as the initial eluent and ethyl acetate added gradiently from 0-25 %. Fractions of 40 ml each were collected, allowed to concentrate/dry under pressure. Column fractions were monitored on TLC (Merck F_{254}), visualizing with UV light (254 nm and 365 nm), *p*-Anisaldehyde reagent or vanillin/sulphuric acid was used as spray reagents. Further purification was carried out on preparative thin layer chromatography (pTLC).

Nuclear magnetic resonance (NMR) spectroscopy conducted includes ID ('H, ^{13}C, DEPT- 90 and DEPT - 135) and 2D ('H, 'H COSY, HMQC and HMBC). NMR spectra was obtained at 300 °K on ARX-400 NMR spectrometers (Bruker, Germany) at the University of Manchester, U.K. Chemical shifts were reported in µ (ppm) and coupling constants in Hz. Infra-red was carried out on attenuated total reflectance (ATR) spectrophotometer. Gas chromatographic analyses were performed on a Hewlett-Packard 6890N GC-MS.

3. Results

3.1 ^{1}H –NMR Spectrum Analysis of Isolated Compound

Compound X: white powder; ^{1}HNMR (500 MHz, J_4-MeOH) 5: 8.03 (IH, s, H-2), 7.36 (2H, d, J = 8.7, H-2^{1}, H-6^{1}), 6.85 (2H, *d, J*= 8.7, H-3', H-5^{1}), 6.31 (IH, d, *J*= 2.0, H-8), 6.18 (IH, d, J = 2.0, H-6). HRESIMS *m/z* 269.0479 [M-H]" (calcd for $C_{15}H_9O_5$, 269.0455).

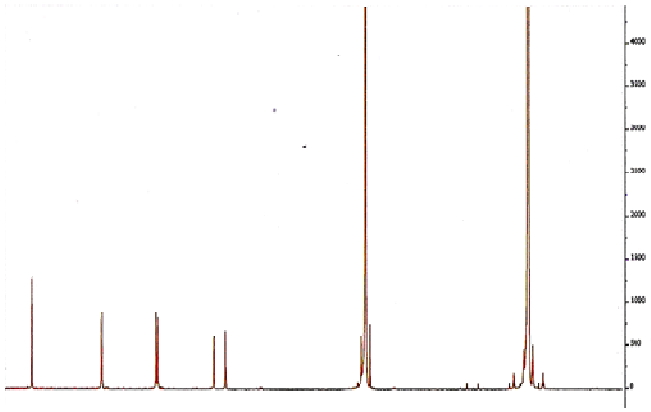
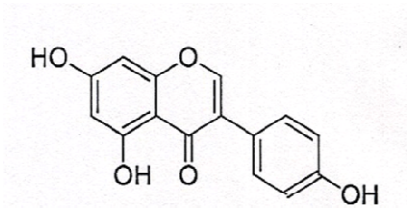


Figure 1: ^{1}H-NMR Spectrum of Isolated Compound

Table 1: NMR Spectroscopic Data for Comparison of Compound X with Literature Values

Position	X	Literature Value
2	8.03s	8.28 s
6	6.18 d (2.0)	6.28 d (2.1)
8	6.31 d (2.0)	6.42 d (2.1)
2'	7.36 d (8.7)	7.32 d (8.8)
3'	6.85 d (8.7)	6.95 d (8.8)
5'	6.85 d (8.7)	6.95 d (8.8)
6'	7.34d (8.7)	7.32 d (8.8)



Figre 2: Proposed Structure of Compound X

4. Discussion

In an attempt to further purify the different extracts, preparative TLC was conducted. Single spots observed on the plates further established the purity. Fractions of methanol were fractionated using TLC method into 80 fractions. Based on TLC-DPPH assay, similar fractions were pooled together by Preparative TLC method to arrive at Compound X. The compound is white with the chemical formula $C_{15}H_{10}O_5$ by HREIMS, molecular formula- *(m/z* 269.0479 [M-H]. The aromatic singlet proton at δ 8.03 in the 'H NMR spectrum indicates that it might be an isoflavone. The two doublet proton signals at δ 7.36 (2H, d, J= 8.7, H-2', H-6'), and 6.85 (2H, d, *J*= 8.7, H-3^{1}, H-5^{1}), indicates the presence of a 4-hydroxy substituted B ring. The other remaining aromatic peak at δ 6.31 (1H, d, *J*= 2.0, H-8) and 6.18 (1H, d, *J* = 2.0, H-8) indicates *Meta* coupled protons in ring A. The final structure was confirmed by comparing the NMR data of Compound with the literature data to be Genistein. The NMR spectrum for Compound was obtained in methanol- δ_4 and the literature values were obtained in DMSO.

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References

Blinov, F.A. Carlson, D., Elyashberg, M. E., Martin, G. E., Martirosian, E. R, Molodtsov, S., & Williams, A. J. (2003). Computer-assisted structure elucidation of natural products with limited 2D NMR data: application

of die StrucEluc system. *Magnetic Resonance Chemistry, **41***, 359-372.

Bross-Walch, N., Kuhn, T., Moskau, D., & Zerbe, O. (2005). Strategies and tools for structure determination of natural products using modem methods of NMR spectroscopy. *Chemistry and Biodiversity,* **2**, 137-177.

Deng, G., & Sanyal, G. (2006). Application of mass spectrometry in early stages of target *based drug discovery. Journal of Pharmaceutical Biomedical Analysis*, ***40** (3), 528-538.*

Guthikonda, R. N., Cama, L. D., Quesada, M., Woods, M. F., Salzmann T. N., & Christensen, B. G. (1987). Modification of natural products to improve their biological properties. *Pure and Applied Chemistry, **59***(3), 455-458.

Houghton, P. J. (2005). Traditional Plant Medicine as a source of new drugs. *In:* Evans W.C. (Ed) *Trease and Evans Pharmacognosy* 15th edn. Saunders*, an imprint of Elsevier* India, Pp.125- 134.

Jaspars, M. (1999). Computer assisted structure elucidation of natural products using two- dimensional NMR spectroscopy. *Natural Product Representative, **16*,** 241-248.

Korfmacher, W. A. (2005). Principles and application of LC-MS in new drug discovery. *Drug Discovery Today*, ***10***(20), 1357-1367.

Meusinger, R. (2006). Spectroscopy Challenge 11. *Analytical and bio-analytical chemistry, **384*.** 1-3.

Neri, P., & Tringal, C. (2004). Applications of Modern NMR Techniques in the Structural Elucidation of Bioactive Natural Products *In:* Corrado Tringali (Ed) *Bioactive Compounds from Natural Sources Isolation, characterization and biological properties* Universitá di Catania, Italy. London and New York, Pp. 69-128.

Noda, I. (2008). Recent advancement in the field of two-dimensional correlation spectroscopy. *Journal of Molecular Structure, **883***(884), 2-26.

Paschal, M. E., Correetero, M. E., Sloving, K. V., & Villar, A. (2002). Simplified screening by TLC of plant drugs. *Pharmaceutical Biology, **40***(2), 139-143.

Pauli, G. (2006). Countercurrent chromatography of natural products CCC (2006). *In: The 4th International Conference of Countercurrent Chromatography*, Bethesda. MD. August 8-11.

Paulo, A., Gomes, E. T, & Houghton, P. J. (1995). New Alkaloids from *Cryptolepis sanguinolenta. Journal of Natural Products, **58***(10), 1485 - 1419.

Paulo, A., Gomes, E. T., Steele, J., Warhurst, D.C., & Houghton, P. J. (2000). Antiplasmodial activity of *Cryptolepis sangninolenta* alkaloids from leaves and root. *Planta Medica*, ***66***(1), 30-34.

Paulo, A., Gomes, E. T., & Houghton, P. J. (1995). New alkaloids from *Cryptolepis sanguinolenta, Journal of Natural Products, **58*,** 1485-1491.

Paulo, A., Jimeno, M. L., Gomes, E. T., & Houghton, P. J. (2000). Steroidal alkaloids from *Cryptolepis obtusa. Phytochemistry, **53***, 417-422

Paulo, A., & Houghton, P. J. (2003). Chemotaxonomic analysis of the genus *Cryptolepis. Biochemical Systematics and Ecology, **31,*** 155-166.

Wagner, H., & Bladt, S (1996). *Plant drug analysis. A thin layer chromatography Atlas.* 2nd edn., Springer Verlag, Berlin Heidelberg New York, 16-67.