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Isolation and characterization of inositol from the ethanolic leaf extract of *Aspilia africana*.

Basil Nse Ita,[a*] Lahai Koroma[b] and Kalilu Kormoh[c]

[a]*Department of Chemistry, University of Uyo, Uyo, Nigeria*
[b]*Department of Applied Sciences, Eastern Polytechnic, Kenema, Sierra Leone*
[c]*Department of Chemistry, Fourah Bay College, University of Sierra Leone, Sierra Leone*

ABSTRACT

Dried leaves of Aspilia africana were subjected to extraction with ethanol. Partitioning of the ethanolic crude extract with petroleum ether, acetone, chloroform and ethanol yielded white crystals in the ethanolic fraction. Chemical and spectroscopic analysis revealed the crystals to be inositol. This is the first report of the presence of inositol in the leaves of Aspilia africana.

Keywords: *Aspilia africana*, inositol, extraction, spectroscopy, x-ray crystallography.

INTRODUCTION

Aspilia africana leaves is widely used in African traditional medicine. Numerous publications exist on its uses in stopping bleeding, for healing wounds and sores, anti-inflammatory properties, antiplasmodial activity, antiulcer effects, antiviral, antifungal, and antimicrobial activity[1-7]. Recently, the plant has been reported to be rich in vitamins[8]. Previously isolated compounds from *A. africana* include lipids- fatty acids[9], essential oils- limonene and α–pinene[10], two flavonoids glycosides; a flavone- 5-hydroxy-3,4,6,8, tetramethoxy-7-glycosyl-1,3 galactoside and thiarubine A[11-12].

Inositol plays an important part in the health of cell membranes, especially the specalised cells in the brain, bone marrow, eyes and intestines. It promotes healthy hair, hair growth , helps in

controlling estrogen levels, and may assist in preventing breast lumps. It is also used for the treatment of liver disorders, disrupted symptoms related to fat metabolism[13-15]. This study discusses the isolation and characterization of inositol from the ethanolic leaf extract of *A. africana*.

MATERIALS AND METHODS

Plant Material

The leaves of *A. africana* were collected from the Eastern Province of Sierra Leone and identified at the Department of Botany, Fourah Bay College, University of Sierra Leone, Freetown. A voucher specimen was deposited in the herbarium of the Department of Botany, Fourah Bay College.

Extraction

The sample was sun-dried and ground into powder using a laboratory mill prior to analysis. All reagents used were of analytical grade, unless otherwise stated.

The powdered leaves was extracted exhaustively with ethanol in a soxhlet apparatus and the crude extract dried. This was re-extracted with 50ml each of petroleum ether(60-80^{o}C), acetone, chloroform, and finally ethanol. The corresponding extracts were allowed to evaporate at room temperature. The ethanol extract on standing gave white needle-like crystals named LK3 . No crystals were obtained from the other extracts. The obtained crystal was subjected to chemical analysis and spectroscopic studies.

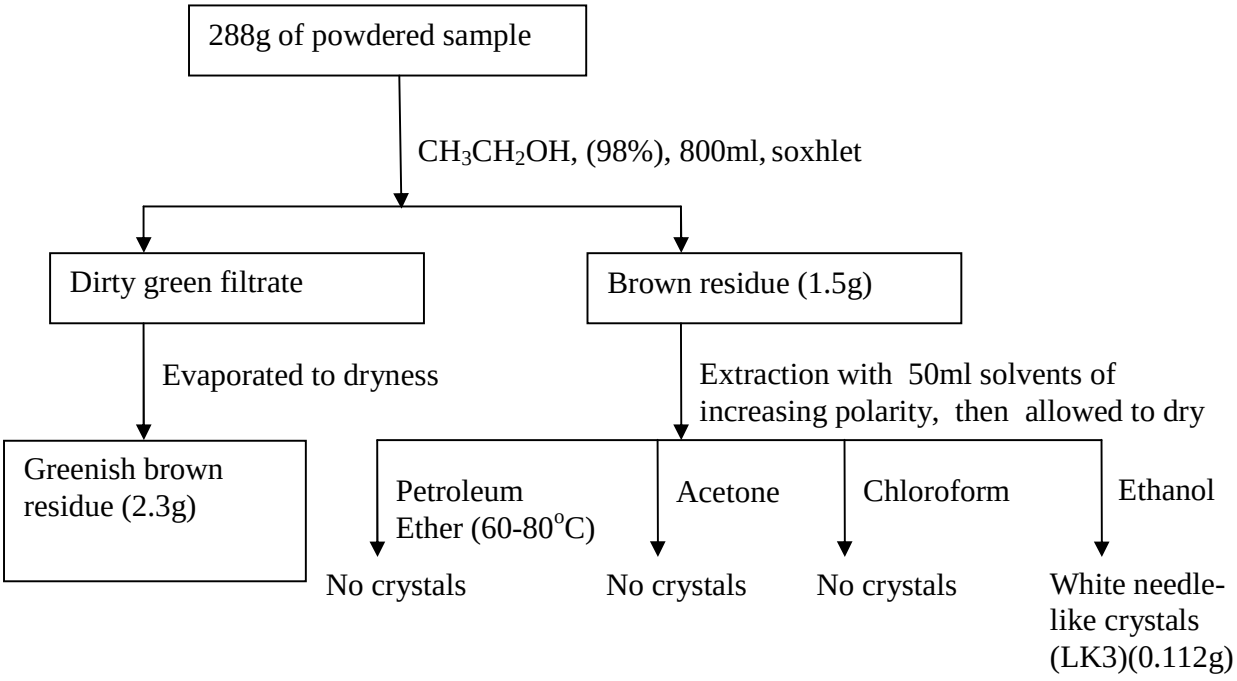


Fig.1: Recapitulative extraction scheme

Instrumental Analysis

Elemental analysis was performed on a Carlo Erba 1106 elemental analyzer. Infrared spectra of sample as KBr pellets were measured with FT-IR spectrometer 5 SXCNicolet DTGS- detector unit. ^{1}H and ^{13}C NMR spectra were measured in D_2O/DMSO and DMSO-MeOH respectively as internal standards using a Bruker AM400Model. The ^{1}H NMR spectra was recorded at 75.035 Hz/cm and J-values are given in Hz. ^{13}C spectra was recorded at 377.3Hz/cm. The crystal data and structure refinement for LK3 were obtained at 173K on a Rigaku AFC6S four – cycle diffractometer with graphite- monochromated Mo-Kα X-radiaton, λ = 0.71073Å. Mass spectra was recorded on positive (fast ion bombardment) FAB spectrometer. Melting points were uncorrected and determined on an electrothermal 9100 apparatus.

RESULTS AND DISCUSSION

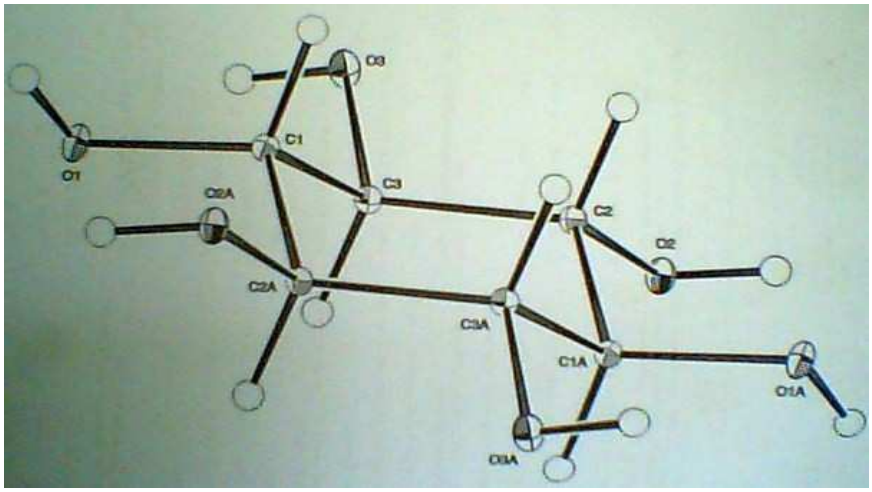
The extraction yields (w/w) for the ground leaves of *Aspilia africana* were 0.52% for the brown residue, 0.80% for the greenish-brown residue and 0.039% for LK3(Fig.1). LK3 on elemental analysis revealed the presence of C, H and O only. It showed a negative test for unsaturation and a positive test for –OH group with a melting point of 248^{o}C, decomposing at temperatures above 330^{o}C, very soluble in water, hot ethanol and aqueous ethanol. LK3 gave very strong peaks at ν_{max} 3407cm^{-1} and 2659.3 cm^{-1} indicative of hydroxide group; weak ν_{max} at 3080-2846.8 cm^{-1} indicating a C-H stretch superimposed upon O-H; ν_{max} 1452-1468 cm^{-1} for C-H bending and cyclohexane ring; a C-O stretch at 995.2 cm^{-1} and a C-H out of plane superimposed on C-H at 827.1 and 765.6 cm^{-1+}[16-17]. The ^{13}C nmr of LK3 gave two distinct solvent peaks corresponding to $\delta_{C\text{-}OH}$ (MeOH, 48.385ppm) and $\delta_{C\text{-}H}$ (DMSO,39.25ppm). In the ^{1}H nmr spectra (DMSO-D_2O;75Hz/cm), four signals were observed: H= 3.1(t) and 4.2(t) ppm due to protons attached at carbons C_{1A} and C_1 interactions with the protons in a trans position at carbons C_{3A} and C_2. The δ values (ppm) at 4.2 (J 0.75), 4.3 (J 0.95) and 4.8 (2H, J 1.60) were all removed by D_2O. This unusual spectrum can be due to steric hindrance and proximity effects of protons attached to carbon and oxygen atoms. However, x-ray crystallography of a compound can be used as a complete analytical tool in the absence of sufficient spectroscopic data, which may arise due to steric hindrance[18].

The mass spectrum of the crystals gave the following peaks interpreted as follows:M/e =185(100%), ($C_6H_{12}O_6$ from X-ray crystallography gave M=180.16). the fragmentation pattern is as follows: [M^+ - D_2O] = 165(8.5%); [M^+ - $2H_2O$] = 149(12%); [M^+ - $3H_2O$] = 131(50%); [[M^+ + O] =201(8.5%); [M^+ + $2H_2O$ + D_2O] =223(8.5%); [M^+ + $4H_2O$ + D_2O] = 277(5%). This fragmentation pattern was arrived at on inspection of the crystal data structure(Fig. 2) of LK3. LK3(cyclitol) has a fragmentation pattern that proceeds with the elimination of water, with the relative abundance of [M^+ - H_2O], [M^+ - $2H_2O$] ions and the axial hydroxyls resulting in the formation of a stable conformation of the molecule[19] and by Mclafferty rearrangement involving a complex fragmentation and dimerization patterns. The crystal – structure analysis of compound LK3 gave structure close to the "ideal" model with bond lengths for [C_1-C_2, C_3-C_1, C_3-C_2] being 1.5241Å, 1.5229 Å and 1.5171 Å respectively (Table1). The expected C-C bond length based on tetrahedral structure is 1.527 Å. The cyclohexane ring of LK3 is chair shaped with [C_1-C_3-C_2-C_{1A}] and [C_2-C_3-C_1-C_{2A}] torsional angles of +56.65^{o} and -56.67^{o} (Table 1). The expected literature value is $\pm$ 60^{o}[20] . From the crystallographic and spectroscopic results, LK3 was identified as inositol or 1,2,3,4,5,6 hexahydroxycyclohexane[17]. Inositol is an

isomer of glucose present in the cell membrane phospholipids and plasma lipoproteins[21]. Antidepressant properties of inositol compared to a placebo, have been reported[22]. Research has reported that a combination of inositol and folic acid was useful in the treatment of erectile dysfunction in men with type 2 diabetes[23]. It has also been implicated in improving ovarian function in women with oligomenorrhea and polycystic ovaries, psoriasis of patients taking lithium for bipolar affective disorders, cancer preventing properties and reduces panic disorders[23-27]. Hence, this plant may prove to be a useful source of inositol.

Table 1: Crystal date, structure refinement and bond angles of LK3

Empirical formula	$C_6H_{12}O_6$
Formula weight	180.16
Temperature	173(2)K
Wavelength	0.71073 Å
Crystal system, space group	
Unit dimension	a=5.0634(9) Å α= 90deg b= 6.6541(11) Å β= 91.84(4)deg c=10.5947(18) Å γ= 90 deg
Volume	356.78(11) A^3
Z, calculated density	4, 1.677Mg/M^3
Absorption coefficient	0.152mm^{-1}
F(000)	192
Crystal size	
Thetha range for data collection	3.62 to 30.50deg
Index ranges	-6<=h<=6, -9<=k<=9, -14<=l<=15
Reflections collected / unique	2863 / 1027 [R(int) = 0.0169
Completeness of 2thetha =30.50	94.1%
Refinement method	Full- matrix least squares on F^2
Data / restraints / parameters	1027 / 0 / 80
Goodness-of-fits on F^2	0.984< 41
Final R indices [I>2sigma(I)]	R1 = 0.0369, wR2 = 0.1126
R indices (all data)	R1 = 0.0378, wR2 = 0.1142
Extinction coeeficient	0.39(4)
Largest diff. peak and hole.	0.515 and -0.334eA^{-3}
Bond lengths [A] and angles(deg)	
C_3-C_2	1.5171(2)
C_3-C_1	1.5229 (11)
C_2-C_1 #1	1.5241 (12)
C_1- C_2 #1	1.5241(12)



CONCLUSION

It is concluded that extraction of leaves of *Aspilia africana*, followed by partitioning gave needle like crystals identified spectroscopically as inositol in the ethanolic fraction. This has been implicated either singly or in combination in the treatment of various ailments, hence the plant serves as a natural source of inositol.

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