D-ARABINO-HEX-1-ENITOL FROM THE INACTIVE FRACTION OF *ACALYPHA WILKESIANA VAR. LACE-ACALYPHA* (MUELL &ARG.)

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Different herbal preparations of *Acalypha wilkesiana var. lace-acalypha* (Muell & Arg.) are employed in traditional medicine for the treatment and management of disease conditions such as wounds, tumors, hypertension, inflammations, skin infections, gastroenteritis and many others. Ethyl 3,4,5-trihydroxybenzoate (ethyl gallate) and 1,2,3-benzenetriol (pyrogallol) had previously been isolated from the active fractions of this plant. However, this present study was done to isolate compound(s) from one of the inactive fractions. Hence, a short silica-gel column chromatography of the inactive fraction (5A) furnished a compound designated as **3** [R_f 0.15; $[n]^{20}_D$ 1.0300]. The structure of **3** has been established to be D-arabino-hex-1-enitol-1,5-anhydro-2-deoxy (1,5-anhydro-2-deoxy-D-enopyranose-arabino-hex-1-enitol) by a combination of ^{1}H NMR, ^{13}C NMR, MS and IR spectral techniques. Compound **3** recorded no antibacterial activity against *B. subtilis*, *S. aureus* and *Ps. aeriginosa.* However, it demonstrated very weak antibacterial activities against *E. coli* and *S. typhi.* which were slightly better than the activity furnished by 5A. Furthermore, it was observed that **3** was inactive against *C. albicans*. Surprisingly, the crude extract and butanol fraction generally demonstrated comparably stronger antimicrobial activities than **3** implying that the purification of the crude extract and 5A did not improve the activity demonstrated by **3**.

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INTRODUCTION

Euphorbiaceae is one the largest families in the plant kingdom[1-3] to which the genus, *Acalypha* belongs.[4,5] Extracts of *Acalypha* species are employed in traditional medicine in countries around the world and a few are well documented in homopathic pharmacopoiea.[6,7]

Acalypha wilkesiana var. lace-acalypha is an ornamental plant which had been introduced to tropical West Africa from other parts of the world and now cultivated as a foliage plant in garden, orchards, greenhouse and parks.[8] Different herbal preparations of this plant are used to treat headaches, fever, skin-fungal infections, mycoses, gastroenteritis,[9-11] breast tumors, wounds, inflammations and hypertension.[12,13]

Prior to this present study, two polyphenols, ethyl gallate and pyrogallol had been isolated from the active fractions of this plant.[14] It is imperative that the inactive fractions should equally be investigated with the aim of isolating any compound(s) therein which would be used to chemotaxonomically mark this species and variety in particular and the genus, *Acalypha* in general respectively.

EXPERIMENTAL

Isolation

Sample 5A (1.5 g, inactive, dirty, viscous yellow substance), a semi-pure residue had been obtained previously from the chromatographic separation of the butanol fraction of the plant.[14] It was purified on a much shorter silica-gel 254 column (Pyrex, USA; 7 g pre-swollen in 100 % toluene; 4 g concentration zone + 6 g separation zone; 11 x 3 cm) by eluting successively with 100 % toluene (110 mL) and 10 % $(CH_3)_2CO$:toluene (60 mL). Fractions of 5 mL each were collected, monitored on silica plates (Merck, Germany) in $(CH_3)_2CO$:toluene:H_2O (10:20:1) and $(CH_3)_2CO$:EtOAc (40:60) using $FeCl_3$/CH_3OH and vanillin-H_2SO_4 as spray reagents. Hence, two sub-fractions coded 5A-I and 5A-II with similar TLC characteristics (R_f values, reaction with $FeCl_3$ reagent or vanillin-H_2SO_4 spray) were bulked.

Further TLC examinations of the sub-fractions in $(CH_3)_2CO$:toluene:H_2O (10:20:1) and $(CH_3)_2CO$:EtOAc (40:60) indicated no materials especially in 5A-I. However, spectral analyses identified 5A-II to be D-arabino-hex-1-enitol-1,5-anhydro-2-deoxy(1,5-anhydro-2-deoxy-D-enopyranose-arabino-hex-1-enitol) and designated as compound **3** (light yellow oil; R_f (0.15); 93 mg). Initially, the refractometer (WAY-15 Abbe, England) was zeroed and the refractive index of **3** was measured at the wavelength (λ) of Na-D line (589.3 nm) at 20.5 °C.[15-17]

Table 1. Antimicrobial screening of crude extract, butanol fraction, 5A and isolate **3** at different concentrations on test microbes in 100 % MeOH

Test microbe	LA 20 mg L^{-1}	BU 10 mg mL^{-1}	5A 5 mg mL^{-1}	3 2 mg L^{-1}	Streptomycine 10 µg mL^{-1}	Nystatin 1 mg mL^{-1}	100 % MeOH
B.subtilis (NCTC 8853)	14	14.5	5	5	23	5	5
S. aureus (NCTC 6872)	14	20	5	5	36.5	5	5
E.coli (NCTC 10764)	5	5	6	7	19	5	5
P. aeruginosa (ATCC 2654)	18	18	5	5	5	5	5
S. typhi (NCTC 5438)	13	7	6	6.5	18	5	5
C. albicans (NCYC 436)	11	5	5	5	5	26	5

Key: The zone diameter recorded is zone of inhibition + size of cup (zone of inhibition +5) mm; LA = Crude ethanolic extract; BU = Butanol fraction; 5A = Semi-pure residue obtained from BU which furnished compound **3**; **3** = D-arabino-hex-1-enitol-1, 5-anhydro-2-deoxy (1, 5-anhydro-2-deoxy-D-enopyranose-arabino-hex-1-enitol); NCTC - National Collection of Type Cultures, Central Public Health Laboratory, Colindale Avenue, London NW9, UK; NCYC- National Collection of Yeast Cultures, UK; ATCC- American Type Culture Collection, Washington, DC

Antimicrobial screening

The micro-organisms used in this investigation included *Bacillus subtilis* (NCTC 8853), *Staphylococcus aureus* (NCTC 6872), *Escherichia coli* (NCTC 10764), *Pseudomonas aeriginosa* (ATCC 2654), *Salmonella typhi* (NCTC 5438) and *Candida albicans* (NCYC 436). They were clinically isolated from specimens of diarrheal stool, abscesses, necrotizing fascitis, osteomyelitis, urine, wounds and vaginal swabs obtained from the Medical Laboratory, University of Uyo Health Centre, Uyo. The clinical isolates were collected in sterile bottles, identified and typed by convectional biochemical tests [18,19] and then refrigerated at – 5 °C at the Microbiology and Parasitology Unit, Faculty of Pharmacy prior to use.

The agar diffusion method was used observing standard procedure with Nutrient Agar CM003, Mueller Hinton CM037 (Biotech Limited, Ipswich, England) and Sabouraud Dextrose Agar (Biomark, India) for the bacteria and fungus respectively. The inoculum of each micro-organism was introduced into each petridish (Pyrex, England). Cylindrical plugs were removed from the agar plates by means of a sterile cork borer (Simax, England) to produce wells with diameter of approximately 5 millimetres. The wells were equidistant from each other and the edge of the plate.[20,21]

Concentrations of 20 mg mL^{-1} of crude extract, 10 mg mL^{-1} of butanol fraction, 5 mg mL^{-1} of 5A and 2 mg mL^{-1} of **3** were introduced into the wells. Also, different concentrations of 10 µg mL^{-1} streptomycin (Fidson Chemicals, Nigeria), 1 mg mL^{-1} of nystatin (Neimeth Plc, Nigeria) and 100 % methanol were introduced into separate wells as positive and negative controls respectively.[22-25] The experiments were carried out in triplicates. The plates were left at room temperature for 2 h to allow for diffusion. The plates were then incubated at 37± 2 ^{0}C for 24 h. Zones of inhibition were measured in millimetre (mm).

Spectroscopic data

The spectroscopic data were obtained on: ES^+-MS on Kratos MS 80, IR on Perkin-Elmer FT-IR 8400S, ^{1}H and ^{13}C NMR on Bruker AC 250 operating 300 MHz for proton and 75 MHz for carbon-13 using CD_3OD as solvent and TMS as internal standard.

RESULTS AND DISCUSSION

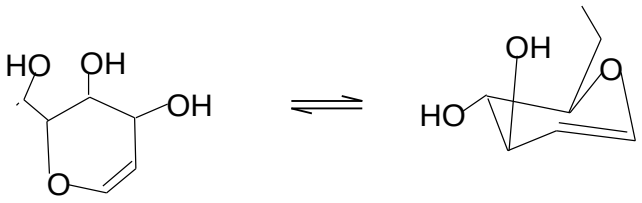
Compound **3**: $C_6H_{10}O_4$; light yellow oil; R_f (0.15); $[n]^{20}_D$ (1.0300); MS [ES^+-MS] m/z (relative intensity): 146 $[M]^+$ - (5.26 %), 128 $[M\text{-}H_2O]^+$ (2.44 %), 115 $[M\text{-}CH_2OH]^+$ (1.27 %), 97 $[M\text{-}CH_2OH\text{-}H_2O]^+$ (8.52 %), 73 $[M\text{-}CH_2OH\text{-}2H_2O\text{-}6H]^+$ (100.00 %) (base peak), 55 $[M\text{-}C_6H_3O]$+(52.83 %) and 29 $[M\text{-}C_6H_9O_3]^+$ (40.32 %) ; IR [FTIR] cm^{-1}: 1061 (C-O), 1653 (CH=CH) and 3526 (OH); ^{1}H NMR δ (ppm): 1.45 and 5.15 (olefinic proton); ^{13}C NMR δ (ppm): 32.76 (methylene-C), 105.13, 105.34 (hydroxylated-C) and 121.22 (C=C).

Elucidation of the chemical structure of compound 3

The determinations of physical parameters are important in identifying compounds. Physical constants such as optical rotation, optical density and refractive index are used in the qualitative and quantitative analyses of substances. Also, these parameters are employed to confirm the purity, identity, integrity of active substances and as well as monitor the progress of reactions.[15-17] The physical examination of compound **3** showed that it was an oily substance. In this study, only the refractive index was measured at the wavelength (λ) of Na-D light (589.3 nm) and a temperature of 20.5 ^{0}C. The measured refractive index of compound **3** is 1.0300. The refractive index of a substance is an indication of the number, type of atoms and chemical groups (species) in the substance. Each atom or group in the substance contributes to its refractivity which

adds eventually to the refractive index of the substance. Furthermore, refractive index can be used to monitor the progress of chromatographic separation by measuring the refractive indices of the effluent solvents employed.[15-17] The structure of **3** was established by a combination of above-mentioned spectroscopic techniques. The obtained MS data were matched with library data of organic compounds.[26]

Hence, compound **3** was identified to be D-arabino-hex-1-enitol-1, 5-anhydro-2-deoxy (1, 5-anhydro-2-deoxy-D-enopyranose-arabino-hex-1-enitol). The ES^+-MS of **3** showed diagnostic fragmented peaks such as $[M]^+$ at m/z 146 (5.26 %) while 126 (2.44 %), 115 (1.27 %) and 97 (8.52 %) represented the losses of water, methylene alcohol and water and methylene alcohol units respectively from the molecular ion. Furthermore, the ion at 73 (100 %) indicated the base peak while ions at 55 (52.83 %) and 29 (40.32 %) were quasi-peaks.[17,27-30] The IR spectrum of the **3** showed diagnostic absorption stretchings at 1653 and 3526 cm^{-1} representing the -CH=CH and -OH functional groups respectively. In addition, the -C-O absorption (ether linkage) at 1061 cm^{-1} was equally very diagnostic. Though, the ^1H and ^{13}C NMR spectra could not readily be used to identify **3** but the ^1H signal at 5.15 ppm indicated the presence of olefinic proton while ^{13}C signals at 105.13, 105.34 and 121.22 ppm showed the presence of hydroxylated-C and C=C (unsaturation) respectively. Compound **3** is presented both in the planar and chair conformations.[31]



Compound **3**.

Antimicrobial screening

The results of the antimicrobial tests displayed in Table 1 show that **3** recorded no antibacterial activity against *B. subtilis*, *S. aureus* and *Ps. aeriginosa*. However, it demonstrated very weak antibacterial activities against *E. coli* and *S. typhi*.which were slightly better than the activity furniushed by 5A. Furthermore, it was observed that this compound was inactive against *C. albicans*. This particular observation was not surprising because fungal strains such as *Candida spp*. limit the permeation of substances because of their integral structures which are pleomorphic and facultative in nature hence, resembling those of higher plants.[32]

CONCLUSIONS

In this study, D-arabino-hex-1-enitol-1, 5-anhydro-2-deoxy has been isolated from the inactive fraction of *A. wilkesiana var. lace-acalypha* (Muell & Arg.). It is expected that this compound would serve as a chemotaxonomic marker for this species and variety in particular and the genus, *Acalypha* in general. However, the isolated compound was generally inactive against bacterial and fungal (candidal) strains.

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References

[1]Dalziel, J. M., *Useful Plants of West Tropical Africa. Crown Agents for Overseas Governments and Administrations*, **1956**, 45.

[2]Sofowora, A., *Medicinal Plants and Traditional Medicine in Africa*. Spectrum Books Limited, **2008,** 85.

[3]Trease, A., Evans, W. C., *Pharmacognosy*. 4th edition, W. B. Saunders Company, **2009,** 54-58.

[4]Oliver, B., *Medicinal Plants in Nigeria I*. Nigerian College of Arts, Science andTechnology, **1959**, 58.

[5]Oliver, B., *Medicinal Plants in Nigeria II*. Nigerian College of Arts, Science and Technology, **1960,** 45-46.

[6]THPUSA. 6th edition, Otis Chapp Inc., **1941**, 65.

[7]HPI. **1971**, *1*, 79.

[8]Burkill, H. M., *The Useful Plants of West Tropical Africa*. 2nd edition, Royal Botanical Gardens, **1985,** 94-96.

[9]Oladunmoye, M. K., *Int. Trop. Med.*, **2006**, *1(3)*, 134-136.

[10]Akinyemi, K. O., Oladapo, O., Okwara, C. E., Ibe, C. C., Fasure, K. A., *Compl. Alter. Med.*, **2005**, *5(6)*, 886-890.

[11]Oloduro, A. O., Bakere, M. K., Omoboye, O. O., Dada, C. A., *Ife J. Sci.*, **2011**, *13*, 23-25.

[12]Bussing, A., Stein, G. M., Herterich, A. I., Pfuller, U., *J. Ethnopharmacol*., **1999**, *66*, 301-309.

[13]Ikewuchi, J., Anyadiegwu, A., Ogono, E. Y., Okungbara, S. O., *Pak. J. Nutr.*, **2008,** *17(1)*, 130-132.

[14]Oladimeji, H. O., Tom, E. U., Attih, E. E., *Eur. Chem. Bull.*, **2014**, *3(8)*, 1-4.

[15]Olaniyi, A. A., *Essential Medicinal Chemistry*. 1st edition, Shaneson C. I. Limited, **1989**, 137-157.

[16]Olaniyi, A, A., Ogungbamila, F. O., *Experimental Pharmaceutical Chemistry*. Shaneson C. I. Limited, **1991**, 78-79.

[17]Olaniyi, A. A., *Principles of Quality Assurance and Pharmaceutical Analysis*. Mosuro Publishers, **2000**, 151-158, 216-217, 264-268 and 443-457.

[18]Gibson, L., Khoury, J., *Lett. Appl. Microbiol*., **1986**, *3*, 127-129.

[19]Murray, P., Baron, E., Pfaller, M., Tenover, F., Yolken, R., *Manual of Clinical Microbiology*. American Society of Microbiology Press, **1995**, 973.

[20]Washington, J., *The Agar Diffusion Method*. In**:** *Manual of Clinical Microbiology*, 4th edition, American Society of Microbiology Press, **1995**, 971-973.

[21]NCCLS. *Performance Standard for Antimicrobial Susceptibility* Test, 8th edition, Approved Standard, The Committee, **2003**, 130 .

[22]Oladimeji, H. O., *Bioactivity Quided Fractionation of Acalypha wilkesiana Muell & Arg*. M. Sc, Thesis, Obafemi Awolowo University, **1997.**

[23]Adesina, S. K., Idowu, O., Ogundaini, A. O., Oladimeji, H., Olugbade, T. A., Onawunmi, G. O., Pais, M., *Phytother. Res.*, **2000**, *14*, 371-374.

[24]Nia, R., *Isolation and Characterization of Antibacterial Constituents from Calliandra haematocephala Hassk and Cissus quandrangularis L.*, Ph. D. Thesis, Obafemi Awolowo University, **1999.**

[25]Oladimeji, H. O., *Chemical and Biological Studies on Cyathula prostrata (L.)Blume*, Ph. D. Thesis, University of Uyo, **2012.**

[26]Lopez-Avila, V., *Org. Mass Spect*., **1987**, *22*, 557.

[27]Beynon, J. H., Williams, A. E., *Thermal Analysis, Techniques and Applications*, Chapman and Hall, **1988**, 76-79.

[28]Millard, R. J., *Quantitative Mass Spectra.* Clapton Moore Press, **1979**, 73.

[29]Constantin, E., Schnell, A., *Mass Spectrometry*. Ellis Horwood Press, **1990**, 141-146.

[30]RSC. *Specialist Reports on Mass Spectrometry*, **1999**, *1*, 57-59.

[31]Morrison, R. T., Boyd, R. N., *Organic Chemistry*, 12th edition, Allyn and Bacon Inc., **1977**, 299-300.

[32]Brown, M. R., *Pharm. J.*, **1975,** *215*, 239-242.

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