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Isolation, Characterization and Antibacterial activity of 4-Hydroxy-2-(1-P-tolylethylidene)-5, 6-dihydrofuro-3-one from the Seeds of *Brachystegia eurycoma* Harms

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Abstract

A new tolylethylidene benzofurone identified as 4-hydroxy-2-(1-p-tolylethylidene)-5, 6-dihydrofuro-3-one was isolated from ethanol extract of the seeds of *Brachystegia eurycoma* Harms. The structure was elucidated using NMR spectroscopy in combination with IR and MS spectral data. The isolated compound inhibited *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Streptococcus fecalis*. This result supports the use of *Brachystegia eurycoma* in phytomedicine for the treatment of diseases and infections as well as healing of wounds in Nigeria.

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Brachystegia eurycoma, Antibacterial activity, Phytomedicine, Bioactive compound

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INTRODUCTION

The consumption of plant extracts in herbal medicine for the treatment of diseases and prevention of infections has grown with tremendous recognition, giving rise to the need to identify the specific compounds that could impart such therapeutic benefits. Phytoconstituent compounds comprise a large group of molecules derived from a variety of plant sources. Many of these compounds are powerful antibiotics and antioxidants and are associated with variety of potential beneficial effects [1, 2]. For example, phytoconstituent compounds may be protective against atherosclerosis and cardiovascular disease and some may have anti-inflammatory, antithrombotic, anti-allergic, antifungal

and estrogenic effects. As part of our contributory effort on the ongoing research on Nigerian vegetation we report herein the isolation, characterization and antibacterial activity of 4-hydroxy-2-(1-p-tolylethylidene)-5,6-dihydrofuro-3-one from the seeds of *Brachystegia eurycoma* Harms.

Brachystegia eurycoma belongs to the family *caesalpiniaceae*. It is a dicotyledonous plant classified as legume and grows commonly along river banks. It also grows on well drained soils. The plant is native to West Africa. It flowers between April and May and fruits between September and January. The fruits are very conspicuous and persistently have gelation properties and impart a gummy texture when used in soups in native cookery in Eastern part of Nigeria [3]. The plant possesses a rough fibrous bark, which peels off in patches and often gives out brownish buttery exudates [4, 5]. Despite the fact that it exudes a gum, it is also used for its timber. The seeds have thin testa, which does not withstand long soaking in water. The leaves make excellent browse material for cattle, sheep and goats [5]. The seeds of *Brachystegia eurycoma* help in softening bulky stool and have been associated with the protection against colon and rectal cancer[6, 7]. The exudate is used in faster healing of wounds. The exudate in right combination with mucin and honey is used for wound healing, prevention of bacterial infection, scar formation and promotes regeneration of hair follicles[7,8]. The seed and stem bark extracts of *Brachystegia eurycoma* have been reported to have marked anti-inflammatory activities at concentrations of 100 and 50 mg/kg body weights in carrageenan-induced acute and formalin-induced chronic inflammatory models in 36 albino rats[9]. The use of *Brachystegia eurycoma* plant in herbal medicine and phytopharmaceuticals for the treatment of wounds, boils and other infections necessitated a probe of its phytoconsituents leading to the isolation of a bioactive compound that exhibited antimicrobial activity against certain strains of bacteria thereby justifying the use of the plant in phytomedicine in Nigeria.

MATERIALS AND METHOD

Experimental

The IR spectra were determined on a Thermo Nicolet Nexus 470 FT-IR spectrometer. The ^{1}H NMR spectra were recorded on a Bruker Avance 400 FT spectrophotometer using TMS as internal standard. Chemical shifts were expressed in parts per million. LC-ESIMS spectra were determined in the positive ion mode on a PE Biosystem API 165 single quadruple instrument; HRESIMS (positive ion mode) spectra were recorded on a Thermo Finniga MAT 95 XL mass spectrometer. Column chromatography was carried out with silica gel (200 – 300 mesh) and to monitor the preparative separations, analytical thin layer chromatography (TLC) was performed at room temperature on pre-coated 0.25 mm thick silica gel 60 F_{254} aluminum plates 20 x 20 cm Merck, Damstadt Germany.

Plant Materials

Brachystegia eurycoma seeds were bought from Umuahia main market in Abia State, Nigeria. The plant seeds were identified and authenticated by Mr. I.K Ndukwe of the Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture, Umudike. Clean and wholesome seeds were selected. The seeds were weighed (1kg) and then decoated by soaking in water for 24 hours. The loosened hull was washed with several changes of water. The dehulled seeds were air-dried and then milled into a uniform and fine powder by a mechanically driven attrition mill. The powdered plant material was dried and kept properly for further use.

Extraction and Isolation of Plant Materials

The powdered seeds of *Brachystegia eurycoma* (500g) was packed into a soxhlet apparatus (2L) and extracted exhaustively with 1000 ml ethanol for 24 hours. The ethanol extract was concentrated using a rotary evaporator at room temperature and left on the laboratory bench for 2 days. The column was packed with silica gel and the extract eluted with different fractions of chloroform, petroleum ether and methanol to obtain the compound. It gave R_f value of 0.78 on Thin Layer Chromatography [using chloroform and methanol (7:3)].

Bioassay

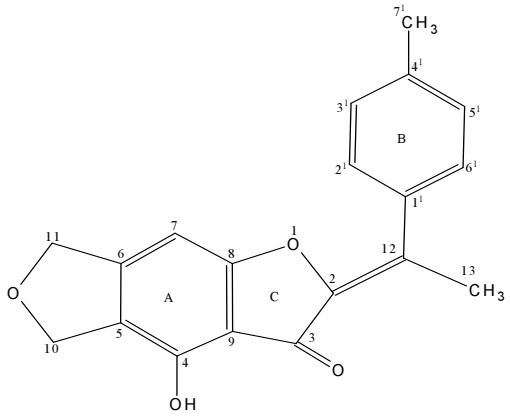
The *in vitro* antibacterial activity of the isolated compound was carried out for 24h culture of three selected bacteria. The bacteria organisms used were *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Streptococcus fecalis*. All the test organisms were clinical isolates of human pathogens obtained from stock cultures at the Central Laboratory services Unit of National Root Crops Research Institute, Umudike, Abia State, Nigeria. With the aid of a single hole punch office paper perforator, circular discs of 5 mm diameter were cut from Whatman No 1 filter paper. The paper discs were boiled in distilled water for an hour to remove any residual preservatives. The boiled paper discs were allowed to drain dry and they were wrapped in aluminium foil and sterilized in an autoclave at 121°C for 15 minutes. They were however used within 48 hours of production. The sensitivity of each test microorganism to the compound was determined using the Disc Diffusion Technique[10,11]. A loopful of each test sample organism was aseptically transferred into the surface of a sterile solid medium, appropriate for the test organism. Using a flamed glass hockey, the innoculeum was spread evenly over the surface of the medium, and then with the aid of a flamed pair of forceps, the extract bearing paper discs was carefully place on the surface of the inoculated medium at some distance from one another. The inoculated plates were incubated for 24 hours in an incubator at 37°C. They were examined daily for growth and for the presence of inhibition zones around the paper discs. The level of sensitivity was determined by the diameter of the inhibition zone as measured with a transparent millimeter rule. The minimum inhibitory concentration (MIC) was determined by comparing the different concentrations of the compound having different zones and selecting the lowest concentration.

Statistical Analysis

All bioassay were replicated three times and means determined[12].

RESULTS AND DISCUSSION

The molecular formula of the compound was established as $C_{19}H_{16}O_4$ based on its HREIMS and NMR data. The IR spectrum of compound **1** (the isolated compound) showed a strong, broad band at 3368.79 cm^{-1} due to O-H stretching typical of phenols. There were absorptions at 1267.00 cm^{-1} and 1070.72 cm^{-1} characteristic of C-O stretching vibrations in the compound. A strong band at 2920.71 cm^{-1} was due to C-H stretching vibration and is characteristic of the hybridization of the carbon which was indicative of a tetrahedral carbon. Absorption due to C-H bending occurred at 1374.34 cm^{-1} for methyl (CH_3) functional group. Absorptions at 1607.43 cm^{-1} and 1460.22 cm^{-1} were characteristic of C=C stretching of aromatic bonds while 1650.00 cm^{-1} absorption was for alkene double bond. For the aromatic ring, out-of-plane C-H bending gave absorption at 721.09 cm^{-1}. The spectrum also showed absorption at 1723.07 cm^{-1} which was indicative of a carbonyl group.



4-hydroxy-2-(1-p-tolylethylidene)-5,6 dihydrofuro-3-one.

Compound 1

Table 1: IR Absorptions of compound 1

IR Absorption (cm^{-1})	Functional group	Compound type
3368.79	O-H	Alcohol
2920.71	C-H	Alkane
1607.43	C=C	Aromatic
1460.22	C=C	Aromatic
721.09	C-H	Aromatic
1723.97	C=O	Carbonyl
1267.00	C-O	Ether
1070.72	C-O	Ether
1650.00	C=C	Alkene

The proton NMR spectrum of compound **1** showed the presence of $-CH_3$ protons (at C_{13}) at chemical shift of δ1.3341. The three protons appeared as a

three-proton singlet peak. The aromatic $-CH_3$ protons also appeared as a three-proton singlet peak at δ2.6462 chemical shift. The two-CH_2 ether protons at C_{11} and C_{10} have their absorptions at δ 3.5255 and δ3.6372 respectively. Each of these $-CH_2$- protons appeared as a two-proton singlet peak. A singlet peak at δ4.3117 was as a result of the –OH proton. The two otho protons of the benzene ring B coupled since they were in the same chemical environment, however, they were split by the two meta protons to give a doublet peak at δ7.3177. The same reverse phenomenon occurred for the meta protons to give another doublet peak at δ7.6362. The only para proton in the benzene ring A gave a singlet peak at δ7.9228.

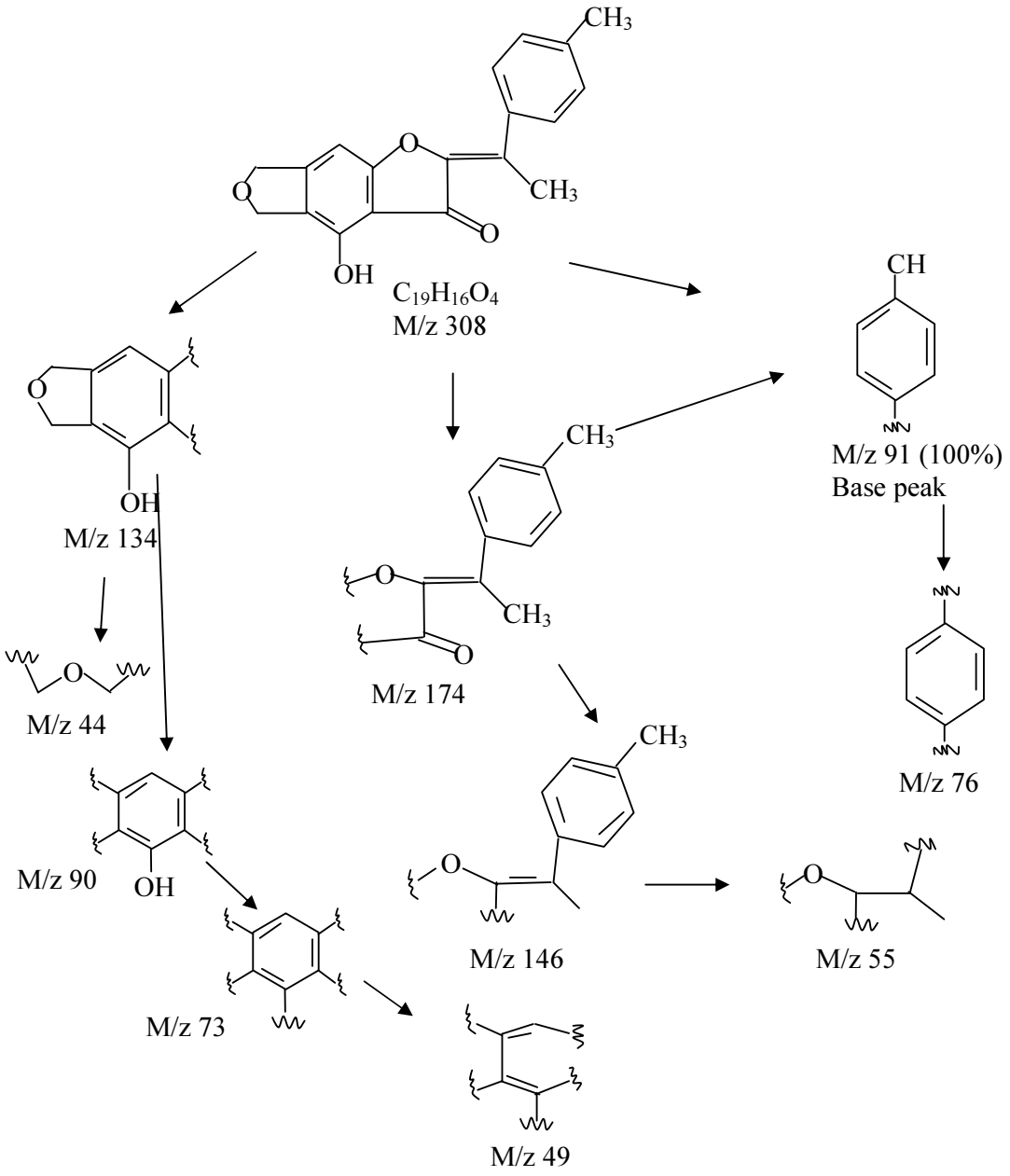


Figure 1: Fragmentation Pattern of Compound 1

Table 2: Proton NMR Chemical Shifts and Multiplicities of Compound 1

Position	Chemical Shifts (δ)	Multiplicity
4	4.3117	1Hs (OH)
7	7.9228	1Hs
10	3.6372	2Hs
11	3.5255	2Hs
13	1.3341	3Hs
2^1	7.6362	1Hd
3^1	7.3177	1Hd
5^1	7.3177	1Hd
6^1	7.6362	1Hd
7^1	2.6462	3Hs

s =Singlet, d = doublet

Form MS data, compound **1** was assigned the molecular mass m/z 308.0703 (M^{0+}) calculated for $C_{19}H_{16}O_4$ (m/z 308) with base peak at m/z 91.0551 calculated for C_7H_7 (m/z 91). The base peak occurred due to the detachment of the phenylmethyl portion of the compound. Other prominent peaks occurred at m/z 44.0379, 49.1919, 55.1786, 73.0733, 76.1001, 134.1110, 146.2011 and 174.0190. The fragmentation pattern of compound **1** is shown in Figure 1.

The results of the antibacterial activity of the compound from *Brachystegia eurycoma* seeds are shown in Table **3**. The results of the bioassay reveal that the compound possesses potent inhibition on the bacteria organisms, namely, *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Streptococcus fecalis*.

Table 3: Inhibitory Effects of Compound 1

Pathogen	Concentration (%)				
	25	50	75	100	MIC (%)
Staphylococcus aureus	6.87	9.30	13.87	17.67	25
Escherichia coli	___	8.87	13.48	16.67	50
Pseudomonas aeruginosa	___	8.67	12.67	15.33	50
Streptococcus fecalis	7.37	9.87	14.00	18.87	25

Figure is in mm and includes the diameter of the paper disc (5 mm). Data are means of triplicate determinations

MIC= Minimum Inhibitory Concentration

= zone of no inhibition

Evaluation of the effect of compound **1** on clinically isolated microbial contaminants of boils, wounds and sores showed varying levels of inhibitory activity on these pathogens (Table **3**). The minimum inhibitory concentration (MIC) of compound **1** is 50-25%. The compound exhibited highest antibacterial activity against *S. fecalis* . The order of activity of compound **1** against the bacteria organisms was: *S. fecalis* > *S. aureus* > *E. coli* > *P. aeruginosa* at 25, 50, 75 and 100% concentrations of the compound (Table **3**). *S. fecalis, S. aureus* and *E. coli* are the common cause of urinary tract infections (UTIs)[13, 14]. *S. fecalis* and *S. aureus* cause inflammation of the genital organs, skin infections and damage sperm cells [15, 16]. The test microorganisms are human commensals and have been incriminated in the infection of wounds[17]. Microbial infection of wounds delays healing which can lead to further tissue injury and damage [17]. These findings justify the traditional use of this plant for wound healing and treatment of infections in traditional medical practice. The plant offers wide-scope utilization as a raw material by pharmaceutical industries for drug formulation. The antibacterial activity of the isolated compound has been verified and authenticated by this research. The compound could be used by pharmaceutical firms for drug formulation. Synthesizing the compound by pharmaceutical industries would help to generate an antibiotic drug with little or no side effects since most inorganic antibiotics are not without health threatening side effects. This research is indeed a breakthrough.

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