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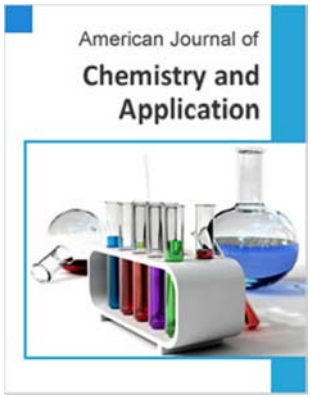

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A Furofuranoid Lignan from the Roots and Some Phytochemicals from the Leaves of *Aspilia africana* (Pers.) C. D. Adams

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Abstract

Aspilia africana (Pers.) C. D. Adams, an indigenous Nigerian plant belongs to the phylum Tracheophyta in the family Asteraceae. Investigation into the hot aqueous leaves extract revealed the presence of phytochemicals such as tannins, saponins, flavonoids and glycosides. Alkaloids were however absent. Examination of the roots also gave a brown oil which was identified by its spectra data such as infra-red, mass, proton and ^{13}C nmr spectra as a furofuranoid lignan (5). Evidence of this comes from the presence of the diagnostic features of the furofurans which include the benzylic protons signal at δ4.50 as a doublet (J=7), the methylene protons at δ3.56 as double doublet (J=5; 10) and the methine protons at δ2.86 as multiplets. The structure of (5) was confirmed by synthesis using ethyl piperonoyl acetate (1). Comparison of the spectra of the naturally occurring compound (5) with that of the synthetic one confirmed that both compounds are the same.

1. Introduction

The search for and use of drugs and dietary supplements derived from plants have accelerated in recent years owing to the phenomenal growth in world population and the attendant depletion in food and drugs [1]. Though plants play significant roles in metabolic activities such as photosynthesis and respiration, the choice for food and drugs is predicated on the presence of phytochemicals in these plants [2]. The importance of these phytochemicals cannot be over emphasized [1] as some of them have been used for the treatment and control of diseases [3, 4] while others have either been shown to inhibit the growth of micro-organisms [5, 6, 8] or be synergistic [9].

Aspilia africana (Pers.) C. D. Adams is an indigenous Nigerian plant found in Etche Local Government Area of Rivers state. It belongs to the phylum tracheophyta of the class magnoliopsida in the family Asteraceae (which is also known as the Compositae). It is a semi woody herb from perennial woody root stock. The height varies from 60cm to 1.5m depending on the amount of rainfall in the zone. The leaves are crowded in capitular heads and the plant possesses bright yellow star shaped petals hence it is commonly known as “wild sunflower”. Some traditional uses of the plant are herein listed below. A decoction of the leaves is used to wash the face and eyes to relieve feverish headache [10, 11]. The leaves infusion is also taken to assist women in child

birth and also to increase milk flow in the mother. A root decoction is taken as oral contraceptive, cough syrup for children and for the treatment of tuberculosis [12, 13, 14]. These uses have aroused interest in investigating this plant with a view to identifying some constituents and phytochemicals present.

Phytochemical analysis of the hot aqueous extract of the leaves of *Aspilia africana* gave tannins, saponins, flavonoids and glycosides; while extraction of the roots with petroleum ether 40-60°C using a soxhlet extractor and subsequent chromatography gave an oil identified as a furofuran namely 2, 4-bis-(3, 4-methylenedioxyphenyl)-3, 7-dioxabicyclo [3, 3, 0] octane (5) by the spectra data such as ir, mass, ^{1}H and ^{13}C nmr spectra. This structure was confirmed by synthesis starting from ethyl piperonoyl acetate (1) in figure 1.

2. Materials and Methods

Infra-red spectra were recorded on Perkin-Elmer 237 Spectrophotometer. Mass spectra were obtained on an AEI, MS 9 double focusing instrument at 250°C and 70eV. Proton and ^{13}C NMR spectra were measured in $CDCl_3$ solution using tetramethylsilane (TMS) as an internal standard on a Varian HA 100 and XL100 spectrophotometers respectively with values given in δ (ppm). Melting point (uncorrected was determined on a Koffler block apparatus. All reagents and solvents were purified before use.

2.1. Extraction of the Leaves of *Aspilia africana*

The plant, identified by Prof. B. Green of the department of Botany, Rivers State University, Port Harcourt, Nigeria, was obtained from Ozuzu in Etche Local Government Area of Rivers State. The shredded leaves and roots were thoroughly washed in flowing tap water and sun dried for 12 hrs and separately ground into powder. Into a 500ml beaker containing 300ml hot water on a hot plate was put 120g of the powdered leaves with occasional stirring using a glass rod. The temperature was maintained at 60°C and the occasional stirring continued during the period of extraction for 10 hrs. The mixture was filtered using Whatman filter paper and the aqueous extract was evaporated on a water bath to give 20ml of greenish-brown liquid used for the phytochemical analysis.

2.2. Phytochemical Analysis

This was carried out using standard methods [15, 16].

2.2.1. Test for Tannins

Into a test tube containing $1cm^3$ of the aqueous extract was added $1cm^3$ of freshly prepared 10% KOH and observed for dirty white precipitate. Into a test tube containing $1cm^3$ of the aqueous extract was added 2 drops of 50% $FeCl_3$ and observed for green precipitate.

2.2.2. Test for Saponins (Frothing Test)

A test tube containing $2cm^3$ of the aqueous extract was vigorously shaken for 2 minutes and observed for persistent foaming.

2.2.3. Test for Flavonoids

To $3cm^3$ of the aqueous extract in a test tube was added $1cm^3$ of 10% NaOH and observed for yellow colouration.

2.2.4. Test for Glycosides

Into a test tube containing $1cm^3$ of the aqueous extract was added $10cm^3$ of 50% H_2SO_4. The mixture was heated in a water bath for 15 minutes and $10cm^3$ of Fehling's solution was added and observed for red precipitate.

2.3. Soxhlet Extraction of the Roots of *Aspilia africana*

A soxhlet extractor was set up and a thimble containing 150g of the root powder was introduced into the extractor and refluxed with petroleum ether 40-60°C for 8 hrs. Evaporation of the solvent on a rotary evaporator gave 4.5g of a dark brown oil which showed mainly as one spot with streaking on a thin layer chromatography plate using 95% CH_2Cl_2 and 5% CH_3CO_2Et. A flash column chromatography of the crude on a silica gel GF 254 eluted with the same solvent system gave 2.4g of a light brown oil.

Yield 2.40g (1.6% Th)

IR (film) (CH) 3100-2920, (aromatic) 1610 cm^{-1}

^{1}H NMR ($CDCl_3$) H-1/5, 2.87m 2H; H-2/4, 4.49 d (7) 2H; H-6a/8a, 3.56 dd (5, 10) 2H; H-6e/8e, 4.00 d (10) 2H; -OCH_2O-, 5.96s 4H; arom., 6.90-6.67m 6H.

Ms m/e 354.1104 M^+ ($C_{20}H_{18}O_6$).

2.4. Synthesis of α-Bromo Ethyl Piperonoyl Acetate (2)

A solution of 190g (0.008 moles) ethyl piperonoyl acetate (1) in 20ml pure chloroform was put into a 150ml round bottom flask. A solution of 1.40g (0.5ml, 0.009 moles) bromine in 15ml dry chloroform was added to the flask dropwise (with cooling when the flask got hot). After the addition, the mixture was stirred at 20°C for a further 3 hr. The mixture was then shaken with water (20ml x 2) and dried over anhydrous $MgSO_4$. Evaporation of the solvent after treatment with charcoal gave yellow oil.

Yield 2.30g (90% Th)

IR (film) (CH) 2980-2880, (COOEt) 1750, (ArCO) 1665, (arom.) 1605 cm^{-1}.

Ms m/e 314 M^+ (2.7%) 316 M^+ (1.8%)

2.5. Synthesis of Diaryldiketo Diester (3)

A solution of 1.40g (0.006 moles) of ethyl piperonoyl acetate (1) in 20ml diethyl ether was added dropwise to a suspension of 0.23g (0.006 moles) of NaH in 25ml dry diethyl ether and stirred for 2hr under nitrogen. 1.90g (0.006 moles) of α-bromo ethyl piperonoyl acetate (2) in 25ml dry

diethyl ether was added drop wise to the sodium salt with stirring and the mixture was refluxed for 5 hr. Stirring was continued for another 5 hr. The mixture was filtered and the solid was dissolved in 50ml of dichloromethane. This was washed with water (40ml x 2) and dried over anhydrous $MgSO_4$. Evaporation of the combined ether and dichloromthane gave an oil which on washing with diethyl ether gave crystalline product recrystallized from ethanol.

Yield 0.42g (30% Th), Melting point 159-161°C

IR (KBr) (CH) 2990-2890, (COOEt) 1725, (ArCO) 1660, (arom.) 1608 cm^{-1}

^1H NMR ($CDCl_3$) arom. 7.84-6.82 m 6H; -OCH_2O 6.02s 4H; CH, 5.45s 2H; $OC\underline{H_2}CH_3$, 3.96q (7) 4H; $OCH_2C\underline{H_3}$, 1.01t (7) 6H.

MS m/e 470 M^+ (5.8%)

2.6. Synthesis of Tetrol (4)

Into a thimble was weighed in 1.90g (4 mmoles) of compound (3) and put in a soxhlet extractor. 25ml of 0.5M (16 mmoles) tetrahydrofuran solution of $LiAlH_4$ was syringed into a 100ml two neck flask prefilled with nitrogen. The mixture was refluxed for 40 hr in an oil bath and then cooled. 20ml 2N HCl was added and stirred for 1 hr and then extracted with CH_2Cl_2 (40ml x 2), washed with water (50ml x 2), dried over anhydrous $MgSO_4$ and evaporated to give brown oil.

Yield 1.10g (69% Th)

IR (film) (OH) 3540-3200, (CH) 2980-2840, (arom.) 1610 cm^{-1}.

^1H NMR ($CDCl_3$) arom., 6.87-6.62m 6H; OCH_2O, 5.86s 4H; ArCH, 4.77dd (4,9) 2H; OH, 3.99 b. s 4H; $C\underline{H_2}OH$, 3.66m 4H; CH, 2.40m 2H.

MS m/e 372 M^+ -18 (5.8%)

2.7. Synthesis of 2, 4-Diaryl Substituted Furofuran (5)

Into a 100ml round bottom flask was put 0.60g (1.4mmoles) of the tetrol (4) in 25ml dry methanol and left standing at 0°C. 20ml of 3M methanolic HCL at 0°C was added to the tetrol solution and the mixture was left at 0°C for 4 hr with occasional swirling. The mixture was extracted with $CHCl_3$ (40ml x 3), washed with saturated $NaHCO_3$ (30ml x 2), then with water (50ml x 2) and dried over anhydrous Na_2CO_3. Evaporation of the solvent gave 0.5g crude product which was chromatographed on a column of silica eluted with 95% dichloromethane and 5% ethyl acetate to give 0.25g of compound (5) as an oil.

Yield 0.25g (43% Th)

IR (film) (CH) 3050-2940, (arom.) 1609 cm^{-1}

^1H NMR ($CDCl_3$) H-1/5, 2.86m 2H; H-2/4, 4.50d (7) 2H; H-6a/8a, 3.56 dd (5, 10) 2H; H-6e/8e, 4.02d (10) 2H; OCH_2O, 5.94s 4H; arom., 6.92 - 6.66 m 6H.

MS m/e 354.1104 M^+ ($C_{20}H_{18}O_6$) 25.2%

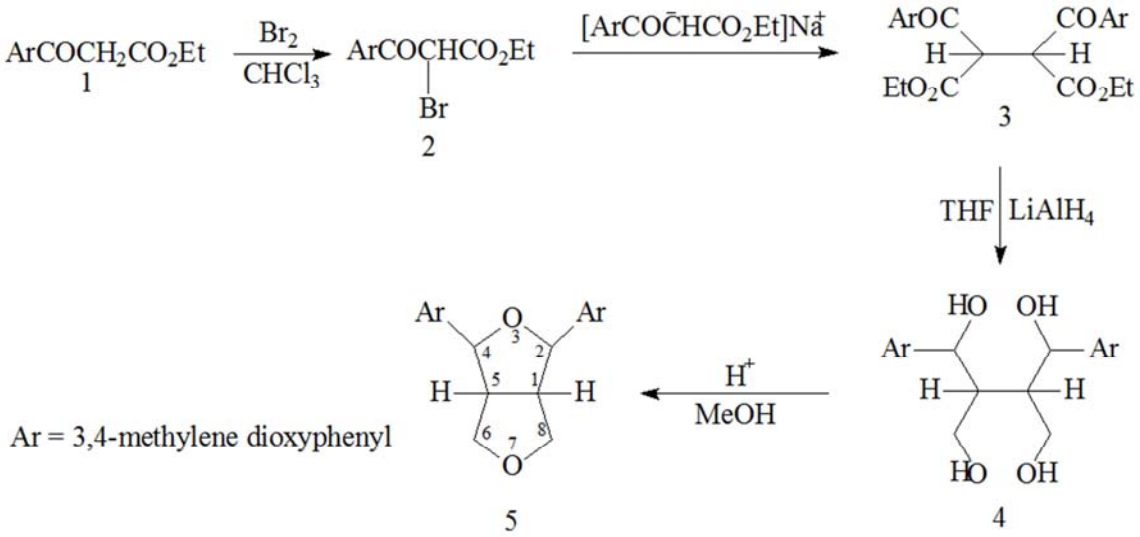

Figure 1. *Synthesis of 2, 4-Diaryl Substituted Furofuran.*

3. Results and Discussions

The phytochemical analysis of the hot aqueous leaves extract of *Aspilia africana* revealed the presence of tannins, saponins, flavonoids and glycosides (Table 1). The presence of these phytochemicals indicates that this plant may be a potential precursor in the development of synthetic drugs. The absence of alkaloids suggests that the plant may not be toxic and this is corroborated by the empirical uses of the plant as cough syrup for children, oral contraceptive and also to increase the flow of breast milk in lactating mothers.

Table 1. *Phytochemical Analysis of the Leaves Extract.*

Phytochemical	Test	Hot Aqueous Extract
Tannins	$FeCl_3$	+
Saponins	Frothing	+
Flavonoids	NaOH	+
Glycosides	Fehlings	+
Alkaloids	Wagner	-

Keys: + Present - Absent

The isolated oil compound (5) from the root gave bands at 3100-2920 and 1610 cm^{-1} corresponding to CH and aromatic group respectively in the infra-red spectrum. In the mass spectrum, it gave a molecular ion M^+ at m/e 354.1104 (25.2%) accurately mass measured to correspond to the molecular formula $C_{20}H_{18}O_6$. The molecular ion fragmented to give other fragment ions. Notable amongst these ions is the one at m/e 284 (18.6%) which resulted from horizontal cleavage of compound (5) and is diagnostic of the 2,4-diaryl substituted furofurans [17] (Figure 2).

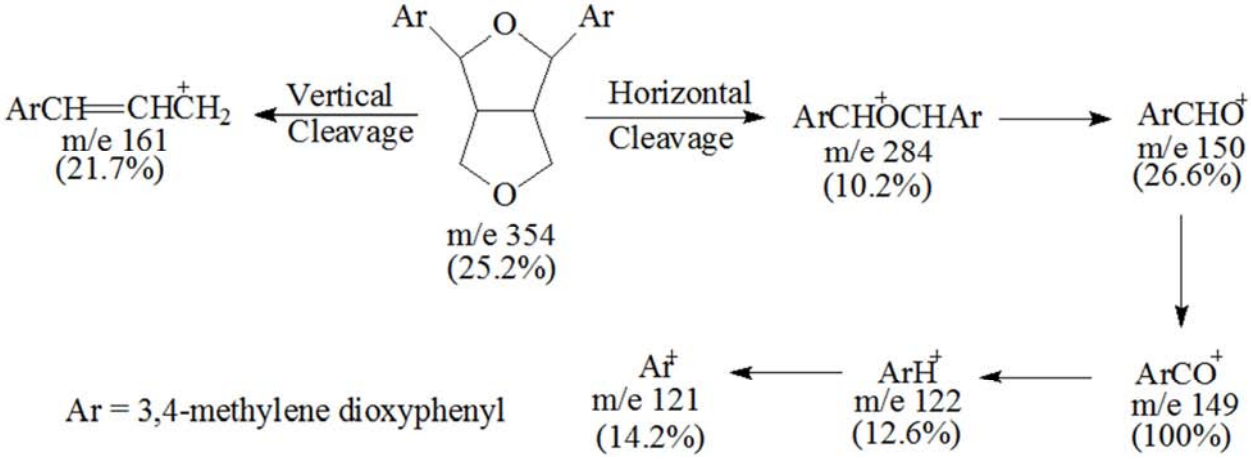

Figure 2. *Fragmentation Pattern of 2, 4-Diaryl Substituted Furofuran.*

A vertical cleavage of compound (5) on the other hand gave the ion at m/e 161 in 21.7% abundance. Other fragment ions given were the ones at m/e 121 and 149 characteristics of the presence of methylene dioxyphenyl moiety [18]. This is confirmed by the positive Labat's test [19, 20]. The aroyl cation at m/e 149 is the base peak.

The proton magnetic resonance spectrum of (5) shows clearly the preservation of the furofuran nucleus with the methine H-1/5 protons appearing at δ2.88 as multiplets being coupled to the benzylic H-2/4 and methylene H-6/8 protons. The H-2/4 protons appeared at δ4.49 as doublets with coupling constant J=7. The methylene protons H-6/8 appeared as two distinct sets of protons, one as axial and the other equatorial. While the axial protons H-6a/8a resonated at δ3.58 as double doublets with J=5, 10; the equatorial protons appeared at δ4.04 as doublet with J=10. The methylene dioxy and aromatic protons resonated at δ5.92 and δ6.90-6.70 as singlet and multiplets respectively (Table 2).

Table 2. *^{1}H nmr of Compound 5.*

Protons	Synthetic Compound	Naturally occurring Compound
1/5	2.86 m	2.88 m
2/4	4.50 d (7)	4.49 d (7)
6a/8a	3.56 dd (5, 10)	3.58 dd (5,10)
6e/8e	4.02 d (10)	4.04 d (10)
OCH_2O	5.94 s	5.92 s
Arom.	6.92-6.66 m	6.90-6.70 m

Spectra run in $CDCl_3$, Coupling constant (Hz) in bracket, Values given in δ (ppm)

The ^{13}C NMR spectrum presented a simple one with C-1/5 which appeared at 55.30, C-2/4 at 87.03 and C-6/8 at 71.51ppm using the criteria established by Pelter and Ward in 1978 [21].

Table 3. *^{13}C nmr of Compound 5.*

Carbons	Synthetic Compound	Naturally Occurring Compound
1/5	55.28	55.30
2/4	87.01	87.03
6/8	71.48	71.51
1^1	134.95	134.96
$2^1/5^1$	106.68	106.70
	108.17	108.19
$3^1/4^1$	147.99	147.97
	148.13	148.09
6^1	119.76	119.80

Spectra run in $CDCl_3$, values given in ppm

Combination of these spectral data deduced the structure of the oil as 2,4-bis(3,4-methylene dioxyphenyl)-3,7-dioxabicyclo[3,3,0]octane (5). This structure was confirmed by synthesis starting from ethylpiperonoyl acetate (1). This was brominated in chloroform to give α-bromoethyl piperonoyl acetate (2) in 90% yield. Knorr's coupling reaction of (2) and the sodium salt of ethyl piperonoyl acetate was undertaken to give the diaryl diketo diester (3). Reduction of (3) with lithium aluminium hydride $LiAlH_4$ in tetra hydrofuran (THF) gave a tetrol (4) in 69% yield. Cyclisation of the tetrol (4) to compound (5) was achieved by reacting the tetrol with 3M methanolic HCl at 0°C for 1hr which on work up gave 43% yield of product. Comparison of the spectra of the synthetic product with that of the naturally occurring compound (Tables 2 and 3) showed similarities in all respect and confirmed that the two compounds are the same.

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