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Iridoid and Caffeic Glycoside Ester from the Leaf And Stem of Stachytarpheta Angustifolia (Mill) Vahl Verbenaceae

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ABSTRACT

Two glycosides were isolated from the n-Butanol soluble portion of the ethanolic portion of the stem and leaves part of stachytarpheta angustifolia. Their structures were elucidated as Iridoid glycoside (1) and a caffeic glycoside ester named as –β- (3^1, 4^1 dihydroxyphenyl)–ethyl-O-α-L-rhamnopyranosyl-(1-3)-β-D-(4-O-Caffeoyl) glucopyranoside (2) by spectroscopic Method.

Keywords: *Stachytarpheta angustifolia, stem leaves, Iridoid, Caffeic glycoside ester, spectral data.*

I. INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects [1]. The research into plants with alleged folkloric use should therefore be viewed as a fruitful and logical research strategy in the search for new drugs [2]. *Stachytarpheta angustifolia* (Mill) Vahl Verbenaceae is a seasonal shrub widely distributed in the subtropical and other temperate regions of the world. The plant is commonly called Wutsiyar bera by the Hausa's while it is known by yorubas as Iru Alangba. The plant is widely distributed in some part of Nigeria [3,4]. The aerial part of *stachytarpheta angustifolia* in Asia and America is boiled and taken traditionally as a remedy against diarrhea, intestinal parasite, skin ulcer and as an Abortificient agent [5]. In Brazil, the triturated fresh leaf of the shrub plant is applied locally for the treatment of ulcer and also taken as a good remedy against rheumatism. The leaves have been reported to be used for the relief of sprain by rubbing the juice on the affected part as well as an adulterant in tea [6]. This plant is reported to contain a glucosidal substance "stachytarphine" which is confirmed to be Abortificient [7]. In some part of the West Africa, Gold Coast according to buntings, the juice from the plant is used as a remedy against eye trouble such as cataract and also applied to sores on children's ear.

In the Northern part of Nigeria, the decoction of the whole shrub mixed with natron is taken as a remedy against dysentery and also for similar condition for horses. The cold infusion of the plant mixed with natron is taken as a remedy against Gonorrhea and other forms of venerable infectious diseases [4, 8].

II. EXPERIMENTAL

Infrared (IR) absorption spectra were recorded using an infrared spectrophotometer. Proton NMR and ^{13}CNMR spectra both (ID and 2D) were obtained using NMR Spectrometer, with the residual solvent peaks as internal standard.

Chemical shift values (δ) were reported in part per million in relations to the appropriate internal solvent standard (TMS). The coupling constant (J-values) were given in Hertz while the HMBC and NOESY are also obtained. The NMR solvent use for this measurement was deutrated methanol [9]

III. PLANT MATERIAL

Stachytarpheta angustifolia (Mill) Vahl (Verbenaceae) was collected from a farm land in Zaria. The shrub was taxonomically authenticated by Musa Gallah of the Herbarium Biological Science Department A. B. U. Zaria. The voucher specimen (No. 900188) was deposited.

IV. EXTRACTION AND ISOLATION

Leaf Portion

The air-dried powdered material (450g) was successively defatted at room temperature with pet ether 60 – 80°c (5 x 600ml) followed by exhaustive extraction using cold ethanol 95% [10]. The ethanolic extract portion (20g) was suspended in water (500ml) and sequentially partition with chloroform, Ethyl acetate and n-Butanol. The various partition portion were concentrated in Vacuum [11].The phytochemical screening of the various partition portions were conducted using standard protocols [12,13,14]. The n-Butanol portion (2.5g) was subjected to silica gel column chromatography and sequentially eluted base on increase in polarity of chloroform, Ethyl acetate and Methanol. The TLC profile of the eluent was monitored using pet- ether, chloroform, Methanol and water (1:1:1:1) and pet ether, Ethyl acetate, Methanol, water (3:7:5:5) [14].

Combination of similar fractions on the basis of TLC analysis affords 450ml fraction of 10ml aliquot. The pooled fraction was hence subjected to repeated gel-filtration using sephadex LH-20 and eluted with Methanol 100% to afford 24fraction of 2ml aliquot. Fraction (6-18) with the same chromatographic pattern were pooled together to afford an amorphous white solid compound 1 [15].

Stem Portion

The air dried portion of the stem material (500g) was extracted with n-hexane and then with hot methanol. (25g) portion of concentrated portion of methanol portion was dissolved in water and partitioned sequentially base on increase in polarity of chloroform, Ethyl acetate and n-Butanol. These were concentrated using rotary evaporator [16].

The n-Butanol soluble portion (2.3g) was subjected to a repeated Gel-filtration using sephadex LH-20, with Methanol 100% as the eluent to obtain 42 portion aliquot of 2ml. Portion (14-32) of the eluent with the same chromatographic pattern were pooled together and concentrated at room temperature [17]. The pooled fractions were further subjected to Gradient elution Techniques using solvent of increasing polarity of chloroform, Ethyl acetate and Methanol. A total of 398 portion of aliquot fraction of 10ml eluent was collected. Portion (58 – 179) of the aliquot with the same chromatographic pattern of two spots on the basis of TLC profile were obtain. These were pooled together and concentrated at room temperature [18].

The concentrated pooled fractions were subjected to (PTLC) techniques using fluka silica gel precoated glass plate (20 x 20cm) with the thickness of 0.25mm. An appropriate concentration solution of pet ether, Ethyl acetate, methanol and water (3; 7: 5: 5) was stabilized in the chromatographic tank [14].

The pooled sample solution was then applied uniformly along 0.5mm line drawn on the plate using capillary tube. The developed plate from the stabilize solution in the tank was allowed to be air dried in the fumed chamber, the band of interest was distinctly scrapped off the back of the plate under UV light 254nm. This was repeatedly washed with methanol, filtered and concentrated at room temperature. The TLC of the amorphous yellow solid affords a single spot using the aforementioned solvent system as compound 2.

Chemical Test

The ferric chloride test, vanillin/sulphuric acid and keddes test were conducted on the two compounds. Solubility and melting point was determine [12, 19,14].

Methylation

5mg of the isolated sample was treated with excess methanol and 2 drops of con. H_2so_4. This was them refluxed overnight and the solution was evaporated to dryness in vacuum. The residue was dissolved in H_2o (O^oc. 5ml) and extracted with CH_2Cl_2 (2 x 10m). The Dichloromethane portion was chromatographed on silica gel and eluted with $CHcl_2$: Methanol (7:3) to obtain a single spot (20, 21).

V. RESULTS AND DISCUSSION

Compound 1.The phytochemical screening reveals the presence of Terpenoids, Steroids, Cardiac glycoside, Saponin and Tannins [12,13,14].

The IR spectrum absorption at (3419.90cm^{-1}), signifies the presence of hydroxyl group. Signal at (3080.42cm^{-1}) shows the presence of (-CH_2^-) present in cyclo hexane with an asymmetric stretch [8]. Absorption at (1986.45cm^{-1}) are for the (C = 0) esters [22].

The (FABMs), gave several fragmentations with Molecular ion peak at $M/_Z$ 429.32 (100%) .The proton NMR shows signal at δH1.5ppm, δH1.8ppm, δH.20ppm all indicating the presence of a methyl proton while signals on δH1.oppm is a characteristics of a methylene proton [17]. Signal at δH4.5ppm suggest the presence of an α – glucosyl anomeric proton arising from a sugar moiety. The large coupling constant of the anomeric proton with a monoglycosidic bond is a characteristic of a β-D-glucopyranose. Signal on δH5.7 is a characteristics proton attributed to the anomeric carbon of the cyclopentano pyran ring system of the aglycones. The (HSQC) correlation shows that δC99.6268 of glucopyranose moiety are couple to those of cyclopentano pyran ring on H/H^1 [23]. Signal observed at δH7.4ppm, a singlet on H-3 is a characteristic proton of an iridoid present on cyclopentano pyran ring system [24]. The HMBC spectrum shows that, the correlation of δH7.4 were observed on δC115.2531 and δC168.0596 respectively [9]. Signals on H3.7 with δC51.6294 suggest the presence of a proton on a carbomethoxy typical of an ester group attached to a cyclopentano pyran ring system as observed above [25].

TABLE I: HNMR AND ^{13}CNMR (CD_3OD.400 MH_Z) SPECTRAL DATA OF COMPOUND 1

H(δ)PPm		J(H_Z)		δ C
1	(5.70) d		1	(94.2549)
3	(7.42) s	(2.0)	3	(152.2531)
6	(3.31) d	(2.6)	4	(115.5908)
7	(3.60) d	(2.5)	5	(38.5408)
8	(2.01) d	(2.4)	6	(40.4164)
9	(1.00) d	(2.4)	7	(48.3752)
10	(1.90)		8	(78.4231)
11	(1.60)		9	(48.5877)
12	(2.00)		10	(48.3114)
1^1	(4.5) d	(8.0)	11	(62.8638)
2^1	(2.6) m		12	(23.2777)
3^1	(3.4) m		13	(168.0596)
4^1	(3.2) m		1^1	99.6268

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5^1 (3.8) m	2^1 74.4259
6^1 (1.8) m	3^1 78.9222
OCH_3 (3.7)	4^1 71.7359
	5^1 77.4788
	6^1 61.6950
	OCH_3 51.6294

TABLE 2: HNMR AND ^{13}CNMR SPECTRAL DATA OF Compound 2 IN (CD_3OD.400 MH_Z)COMPOUND 2 δ C

H(δ)ppm		
2 (7.09)	(s)	1 (127.82)
5 (6.80)	d(8)	2 (114.87)
6 (6.75)	d(8)	3 (146.28)
7 (7.54)	d(16)	4 (148.16)
8 (6.25)	d(16)	5 (144.82)
2^1 (6.65)		6 (123.35)
5^1 (6.50)	d(8)	7 (146.98)
6^1 (6.69)	d(8)	8 (115.39)
7^1 (2.80)	m(8)	9 (168.46)
3.65	m(8,9)	1^1 (131.65)
8^1 (3.84)	m(8,9)	2^1 (116.46)
1^{11} (4.80)	d(7.5)	3^1 (144.82)
2^{11} (3.56)		4^1 (144.20)
3^{11} (3.88)		5^1 (116.67)
4^{11} (4.80)	t(9.5)	6^1 (121.41)
5^{11} (3.42)	m	7^1 (36.72)
6^{11} (3.58)		8^1 (70.56)
(3.51)		1^{11} (103.16)
1^{111} (5.30)	d(1)	2^{11} (74.03)
2^{111} (3.70)	dd(1.2,5)	3^{11} (76.19)
3^{111} (3.30)		4^{11} (70.56)
4^{111} (3.13)	t(9.5)	5^{11} (72.50)
5^{111} (3.39)	m	6^{11} (62.50)
6^{111} (1.05)	d(6)	1^{111} (104.37)
		2^{111} (70.75)
		3^{111} (70.56)
		4^{111} (72.40)
		5^{111} (68.80)
		6^{111} (18.48)

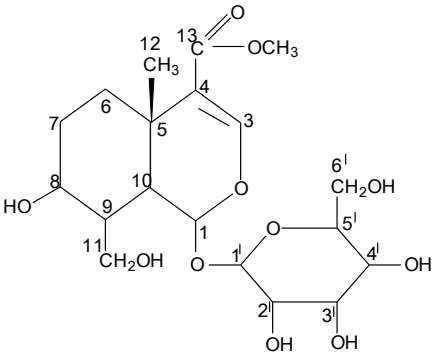
The ^{13} CNMR spectrum of (compound 1) exhibited a total no of 19 carbons, 13 of it corresponding to an aglycone while six to a sugar moiety. This also exhibit 3 quaternary carbons on C – 4, C – 5 and C – 13. The total number of 8 (– CH –) group was observed with 3 (- CH_2 -) group on C-6, C – 7 and C – 3 respectively [26].

Signal at δC168.0596 on C – 13 is a characteristic of a carbonillic carbon coupled to δC51.6294ppm, a methoxy group to form an ester. The HMBC has shown that the ester linkage was to δC115.5909pp, a quaternary carbon of cyclopentano pyran ring system of aglycones. Signals on δC99.6266ppm, δC74.4259, δC78.9222ppm, δC71.7359ppm, δC77.4788ppm and δc61.6950ppm are all characteristic carbon signal for glucopyranose unit. The β – anomeric configuration for the glucose was judged from its J value coupling constants (4.5). The HMBC and its NOESY correlations between C – 1/H – 1, H – 1/C – 1^1 and H – 1/C – 1^1 suggested that the β – glucopyranose unit is attached to (C – 1) position of aglycones unit [27].

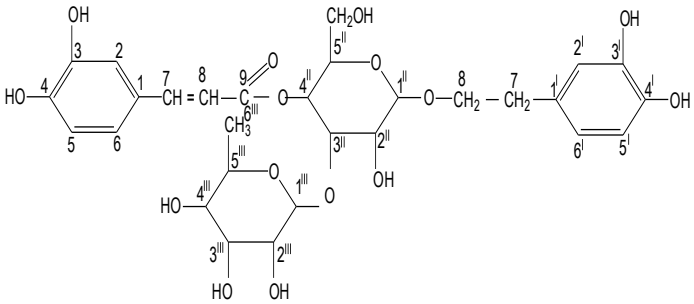
In conclusion, the (FABMs) suggested the molecular weight for compound (1) as 430 $[M-H]^+$ with molecular formular as C_{19} H_{26} O_{11}. The HNMR spectral data has enable us to ascertain that compound 1 is resolved as two ABX systems. One of the residue as an aglycone moiety, while the other as the glucopyranose unit [9].

The ^{13}CNMR has assisted us to ascertain the anomeric carbon position of the sugar moiety to be β-origin. The quaternary carbon couple to cyclohexane ring was observed and the ester linkage of aglycones was ascertained. The comparative studies of the ^{13}CNMR resolves that the position of the sugar unit to be – β– D glucopyranose. Thus compound 1 is identified as an Iridoid glycoside

(1)



(2)



Compound 2: The I.R Spectrum displayed absorption due to hydroxyl at 2447cm indicating an hydroxyl functionality. The absorption at 2954cm indicate a (C – H / stretch) for a methyl group while peak at 14626cm (C=C) indicate several bands on Aromatic rings with many peaks [9] Bands on 1462cm^{-1} corresponds to (C=O) an ester group without an enol ether system.

(FABMS) displayed the following intensive fragmentation M/z 83,8936, 96.3143, 133.3942,

184.9125, 234.0915, 325.4634, 428.5422, 485.2282, 551.3470, 577.1345, 368.3165, 677.4815, 635.4830, 734.5628, 817.2214, 834.9410 and 967.5227 with M/z (100%) at 635.4830[27].

The proton NMR shows signal at δH1.05ppm typical of a tertiary methyl group on H-6^{11}. The signals on δH6.50ppm, δH6.80ppm, δH6.25ppm, δH6.65ppm and δH6.56ppm are all characteristics of an aromatic proton while those on δH-1 and δH-1 with δH4.50ppm and δH5.30ppm are characteristics of sugar protons i.e Glucose and Rhamnose [28]. The signal found on δH1. 05ppm is a major signal found on Methyl group of a Rhamnose sugar δH-6" while the Multiplete signal on δH2. 80ppm are characteristics of an α and β Methylene proton assign to H-7^1 and H-8^1 of a Benzene ring Signifying the presence of a vinylic proton [29]. Signal at δH4.50ppm and δH4.80 ppm on H- 1^1 and H-4" are characteristics of sugar proton which is in conformity with a β– Configuration of an anomeric protons. The glucosyl anomeric proton on δH4.80ppm are linked to caffeoyl moiety by a glycosidic bond [30].Signal at δH5.30ppm on H-1^{111} is an anomeric sugar proton with low coupling constant, a characteristic of an α linkage probably a Rhamnose. The high field signal of δH1.05ppm on H-6^{111} is a characteristic of a methyl group attached to rhamnose sugar.

The methylene proton on H – 8^1 with δH3.84ppm is clearly distinctive by the irradiation of the anomeric proton on H – 1^{11} which modifies the triplet proton at H-2^{11} with δH3.56ppm. This later collapses to produce H-3^{11}, the irradiation of triplet at δH4.80ppm which is reflected by the modification of the complex signal on H-5^{11} with δH3.42ppm. (31). (H – H) COSY shows the coupling of the methyl proton with a sugar proton at δH3.39ppm suggesting that the methyl proton is part of the sugar moiety, a rhamnose , Suggesting that the two sugar are glucose and rhamnose [32]. The signal at δH3. 70ppm from the (H – H) Cosy is coupled to the proton at δH5.30ppm and from this observation we now deduce that the Signals are assigned to H-2^{111} of rhamnose [33].

In CNMR, peaks were assigned on the basis of Chemical shift consideration and comparism with data for glucose and rhamnose. The ambiguity between the two close chemical shift on C – 1^{11} and C - 1^{111} was attributed to δC103.16ppm [27,33]. ^{13}CNMR spectrum was found to exhibit 29 Carbon signals, 9 corresponding to Caffeoyl Moiety, 8 to aglycone, 6 to glucose while 6 to rhamnose sugar respectively [34]. This also shows that 26 out of 29 are Methyl group while 3 of them are methylene groups.

Signals observed at δC146.1605ppm, δC148.0262ppm, δC144.7106ppm on C,-3, C - 1 and C – 5 are characteristic carbon of aromatic nucleus. Those at δ C115. 2093ppm and δC117.1120ppm indicate the presence of an – α and β Vinyl Carbon on δC168.2885ppm which exhibits the presence of a Carbonillic Carbon on C – 9. The hydroxyl substitute on C-3 and C – 4 are similar to the corresponding – β – (3^1, 4^1 dihydroxy phenyl on the aglycones Unit [26,34]. The deshielding effect on C- 3^{11} with respect to the glucose must be attributed to C-1^{111} as rhamnose (1 →3) glucose bond (+δ) effect and to β – caffeoyl effect (-d) effect. The coupling constant of the anomeric carbon of glucose and rhamnose are completely in consistence with the – β – configuration for the former and α for the latter [33,35]. Signals at δC36.72 ppm on C – 7^1 are similar to the corresponding – β – (3^1, 4^1 dihydroxyphenyl) on δC144.82ppm of C – 3^1 and C - 4^1[24].

In conclusion, the FABMS has suggested the molecular formula as C_{29} H_{36} O_{15} with an accurate Molecular weight of 624.594. The proton NMR Spectral data has enabled us to resolve the Aromatic proton as two ABX systems. One belonging to Caffeic acid substitute, a Caffeoyl moiety while the other as 3,4 dihydroxyphenyl ethyl here after designated as aglycones [9].

The CNMR has assisted us to ascertain the anomeric position of the glucose moiety as a – β – Origin from the coupling constant of its anomeric position at δH4.80ppm, while the rhamnose Unit Conforms with those of α – L- rhamnopyranoside. The nature of sugar was confirmed by the comparative data studies of ^{13}CNMR for caffeic glycoside to be – β – D – glucopyranoside. On the basis of this, It was determine that, the rhamnoside to be (- O - α – L- rhamnopyranosyl) while those of the glucose to be (- β – D- glucopyranose). The two sugars are linked to each other on C – 1^{11}←→ C – 3 with glycosidic bonds. Thus compound 2 is named as – β – (3^1, 4^1 – dihydroxyphenyl) ethyl- O - α - L – rhamnopyranosyl (1→ 3) – β – D – (4 – O – Caffeoyl) – glucopyranoside.

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