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Three Iridoid Glycosides from the Root Extract of Stachytarpheta Angustifolia Mill Vahl Verbenaceae

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Abstract

Three iridoid glycosides were isolated from the n-butanol fraction of the root extract of Stachytarpheta angustifolia (verbenaceae). Their structures were elucidated by using a combination of 600MHz/150 MHz ID and 2D NMR techniques (COSY, NOESY, DEPT, HSQC and HMBC) and by FABMS and HRESIM. Compound 1 was characterized as Citrifolinoside, (2) as Serratoside and 3 is determined as 6 – O – (3 " – O – trans – cinnamoyl) - α – L – rhamnopyranosyl catalpol heptaacetate.

Keywords: Stachytarpheta angustifolia, n-butanol, root extract, Iridoid glucosides

Introduction

Iridoid glycosides are large group of naturally occurring Monoterpeniod with a glucose moiety attached at C-1 in the pyran ring (Song et al, 2006). They occupy an important position in the field of natural product chemistry and biology, as they provide a structural link between terpenoids and indole alkaloids and as well display a broad spectrum of biological activities (Sticher, 1977). Iridoids are useful phytochemicals in the number of folk medicinal plants and many of them poses significant biological and pharmacological activities, some of them are chemo taxonomically useful as markers of genus in various plant families. Stachytarheta angustifolia is a shrub of about 5 ft high with a soft cylindrical bark. They are mostly simply or slightly branched often rather succulent with pale blue flowers. They are mostly distributed in the tropical, sub-tropical regions and other part of the world (Dalziel, 2000).

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The triturated fresh root of the plant is applied locally for the treatment of ulcer and also taken as a good remedy to reduce blood pressure as well as an anthelmintic agent. The infusion of the root extract with natron is used to relieves depression as well as reduces fever. The shrub plant is reported to contain a glycosidal substance "Stachytarphine" which is reported to be abortificient (Watt and Breyer-Brandwijk, 1962). The juice from the plant is use as a remedy against cataract and also applied on children's ear. Decoction of the whole plant is used as an Emmenagogue agent while the leaf is taken as a Cholagogue agent and also as remedy against gonorrhea and other related venerable infections (Dalziel, 2001; Jinju, 1990). Literature review reveals the presence of Citrifolinoside, Macrophyloside, Triterpenes, Friedelin, Stigmasterol, Ursolic acids and Oleanolic acid in the genus of this family. Although, no compound have been reported to be isolated from the root of this plant as well as its ethno medicinal properties. In this report, we describe the extraction, isolation and structural elucidation of three Iridoid glycosides from the n-butanol root extract of Stachytarpheta angustifolia extract using standard chromatographic and spectroscopic techniques.

Experimental Section

General Experimental Procedures

Optical rotations were measured using a Perkin –Elmer model 341 LC spectrometer at room temperature. IR spectra were recorded on spectrophotometer shimadzu 8400s. Melting Points were determined on XT4A Apparatus and results are uncorrected. ^{1}H NMR and ^{13}C NMR experiments were performed on Bruker spectrometer 600 MH_Z for ^{1}H and 150 MH_Z for ^{13}C NMR. NMR spectra were referenced to the CD_3OD solvent signals at $\partial 3.30$ (H^{l}) and 49.00 (^{13}C) with TMS as an internal standard. Chemical shift values (∂) were reported in parts per million (ppm) in relation to the appropriate internal solvent standard (TMS). The coupling constants (J-values) were given in Hertz, HRESI –MS was measured on a mass Autospec – ultima – TOF spectrometer. TLC was carried out on plates precoated with RP-18 gel (merck) and silica gel F_{254} (Qingdao Marine Chemistry Ltd). Spots on the plates were visualized by spraying with 10% H_2SO_4 followed by heating in oven. Column chromatography was performed on silica gel 60 (0.040 -0.0653 mm), column (40 – 63μm, 310mm α 15mm i.d). GC analysis was performed on a Shimadzu GC-2010 gas chromatographic equipped with an H_2 flame ionization detector and a DB-5 quartz capillary column (30cm x 0.25mm x 0.25 μm).

Gel filtration technique was carried out on sephadex $LH_{20,}$ TLC visualization was by UV absorption at 254mm. All solvents were distilled prior to use.

Plant Material

The plant Stachytarpheta angustifolia was collected locally from Basawa, a village outskirt of Zaria in Kaduna state of Nigeria in the month of august, 2013. Botanical identification was performed at the herbarium section of the department of biological science, Ahmadu Bello University Zaria, Nigeria and voucher No. 900188 was obtained. The fresh plant material was carefully separated into different parts, the leaf, the stem and the root.

Extraction and Isolation

The air-dried portion of the root was made into finely powdered material using pestle and mortar. The root powdered material (1.25kg) was exhaustively extracted at warm temperature (25 ± 5^0) with methanol (6 liter x 4times) and the methanol extract was concentrated in vacuo to give a residue (210g). Water (300ml) was added and it was then partitioned sequentially with n-hexane, chloroform, ethylacetate and n-butanol. The solvents from the various portions were recovered using rotary evaporator. The n-butanol fraction (10g) was solubilized in (CH_3OH) (15ml) and precipitated in di ethyl ether (4x250ml) yielding 4.1g fraction. The mixture obtained was concentrated and then suspended in water dialysed for 2 days and lyophilized, yielding crude mixtures of glycoside from TLC profile. An aliquot (2.3g) of the mixture was fractionalized by column chromatography over sephadex LH_{-20} and then re submitted to a repeated MPLC column chromatography on silica gel 60 (15-40 um) using as eluent $CHCL_3$: CH_3OH : H_2O (3:3:1, 6:4:1 and 8:5:1), affording compound 1 (21mg), 2(25mg) and 3(18mg) respectively.

Acid Hydrolysis for Compound 1

Solution of compound 1 (5mg) in 2M Hcl MeOH (4:1, 5ml) was reflux at 90^0C for 6hrs, after cooling, the reaction mixture was diluted to 20ml and extracted with CH_2Cl_2 (3x2ml). The aqueous layer was concentrated to an appropriate volume (1ml) and examined by TLC (Silica gel) with a solvent system $CHCl_3$ /MeOH/H_2O (65:35:10) for sugar analysis. R_f value of D-glucose was recorded.

The remaining aqueous layer was concentrated to dryness to give a residue and dissolve in pyridine (1ml), and then L-cysteine methyl ester hydrochloride (2mg) was added to the solution. The mixture was heated at 60^0C for 2 hr., equal volume of acetic anhydride was added, followed by heating at 90^0C for another 2hr. The solution was then concentrated to dryness and taken in MeOH (0.5ml), which was analyzed by GC (column: DB-5 quartz capillary column (30m x 0.25mm, 0.25μm), H_2 flame ionization detector column temperature: 160-280^0C programmed increase: 5^0C/min, carrier gas: N_2 (1.5ml/min), injector and detector temperature: 280^0C, injection volume: 1μl, split ratio: 10/1. The derivative of D-glucose was detected with R_f (mm):23.89 and 28.07. The standard sugar was also subjected to the same reaction and GC analysis under the same condition as above was observed (Lan, et al., 2009)

Acid hydrolysis and GC Analysis for Compound 2 and 3

The solution of compound 2 and 3 (4.0mg) each in methanol (25ml) was treated with 3N Hcl (15ml) and stirred at 80^0C for 5hrs. Upon drying with a flow of nitrogen, the residue was dissolved in (-2) -2 – butanol (0.5mL) and a drop of trifloroacetic acid were added. The solution was in each case transferred to an ampoule which was sealed and heated at 130^0C overnight until complete butanolysis. This was taken to dryness, the resultant residue was reacted with hex methyl disilazane /chlorotrimethylsilane /pyridine (1:1:5, 0.1ml) for 35min at room temperature. The solution was centrifuged and the supernatant layer (1 μL) was analyzed by GC using HP-5 column. The injection port and detector temperature was set at 200^0C and 220^0C. A temperature gradient from 140 - 200^0C at 1^0C/min was applied. Four peaks were detected from the hydrolysate at 37.45, 37.61 and Authentic standards were prepared in a similar manner from commercially available D-and L – glucose which gave rise to peaks at 37.43 and 37.51 (Furniss, 1989).

Determination of Sugar Compounds in 2 and 3

A solution of compound 2 and 3 each (6mg) in H_2O (2ml) and 2N aqueous solution of CF_3 COOH (5ml) were refluxed on a water bath for 3hrs. After this period, the reaction mixture was diluted with H_2O (20ml) and extracted with CH_2Cl_2 (4x5ml). The combined CH_2CL_2 extracts were washed with H_2O and then evaporated to dryness in Vacuo. The sugars were analyzed by silica gel TLC by comparison with standard sugars with those in 2, 3 and the TLC of R_f (rhamnose) 0.50 and R_f (glucose) 0.31 was obtained.

Furthermore, the residue from the sugars were dissolved in anhydrous pyridine (100 μL), and L-cysteine methyl ester hydrochloride (0.06 mol/L) was added. The mixture was stirred at 60^0C for 1hr, and then 150 μL of HMDS –TMCS (Hexamethyldisazane - trimethylchlorosilane, 3:1) was added. The mixture was stirred at 60^0C for 30min. The precipitate was centrifuged, and the supernatant layer was concentrated under N_2 stream. The residue was partitioned between n-hexane and H_2O (0.2ml each) and the n-hexane layer (1 μL) was analyzed by GC. L- rhamnose and D-glucose by co-injection of the hydrolysate with standard silylated samples to give single peaks at (18.73min) for D- glucose observed in 2 and 3while (13.48min) for L- rhamnose in compound 3 only (Mohammed, et al., 2003).

Results and Discussions

Extraction of the root extract of S. angustifolia followed by an extensive column chromatography of n-butanol portion of the extract on silica gel and purification over sephadex LH_{20} resulted in the Isolation of compound 1, 2 and 3. Compound **1** was obtained as an amorphous brown solid. The FABMS established a Molecular formula of $C_{26}H_{28}O_{14}$ as confirmed by the high resolution positive FABMS as ([M – H] at M/Z – 563), as well as from its ^{13}C NMR and DEPT NMR data. The IR spectrum indicated the presence of an α, β – unsaturated ϒ – lactone ring at (1750cm^{-1}), an iridoid and ether system conjugated with an ester carbonyl group at (1710, 1642cm^{-1}), a Para substituted phenyl group at (1620, 1517, 820 cm^{-1}) and the presence of hydroxyl groups at 3450cm^{-1} (Silvestein, 1991; Jiang-ming et al., 2003). The ^{1}H NMR spectrum exhibited a doublet (J = 2.0 H_Z) for the characteristic H – 3 proton of an iridoid at δ_H 7.41, a singlet at δ_H 3.74ppm could be attributed to a carbomethoxy group while two doublets with signals (J = 2.5H_Z) each for C – 6 and C – 7 could be attributed to an epoxy protons on δ_H 4.03 and δ_H 3.9ppm. The signal with a doublet (J = 8.0H_Z) for H – 1' attributed to proton on δ_H 4.43ppm suggest, that the cyclo pentano pyran ring system and the sugar moiety are identical to those of the 6β, 7β – epoxysplendoside (Shengmin et al., 2001 ; Kalpoutzakis, et al., 1999). The two pair of proton doublet (J = 8.4H_Z) each at δ_H 6. 87 and δ_H 7.68ppm exhibited the presence of a Para – hydroxyl phenyl group as supported by the values obtained in ^{13}C NMR Spectrum (δc 127.5/C-1c, δc 134.6/C – 2", 134.8/C – 6", 117.08/C-3", 117.04/c-5" and 162.5/c – 6" (Silvestein, 1991).

The ^{13}C NMR spectrum of compound 1(table 1) exhibited a total of 26 carbon atom signals with Ten carbon atoms representing the aglycone, one to methoxy group at δc 51.7, six to the glucopyranose moiety at (δc 99.4/C -1'; 74.5/C-2'; 78.4/C-3'; 74.2/C – 4'; 77.4/C-5' and 62.5 an oxy – methylene attributed to C – 6' (Jun-mian, 2008). The β – anomeric configuration for the glucose moiety was judged from the large J_H coupling constant of J = 8.0 H_Z (Ilyas, et al., 2014). The presence of an oxygenated methine carbon was observed at δ_c 70.2/C - 10 and a single olefinic Carbon atom signal at δ_c 144.04/C – 13 is an indicative of a characteristic iridoid with five membered spiro – lactone ring (Shengmin et al., 2001; Kemp, 1991). The DEPT spectrum exhibited the presence of an – oxymethylene on C – 6', oxygenated methine carbon at C – 10, the presence of 2 – epoxy carbon signals at C – 6 and C – 7. The presence of a single carbonyl signal was observed on C – 14, a methoxy signal on δc 51.7ppm, methylene signals on C – 3" and C – 5", aromatic carbon signals on C – 1", C-2" and C-6" (Jun-mian, et al., 2008). The presence of para substituted hydroxyl group of benzene was observed at C – 4" while an olefinic carbon atom was observed at C – 13 respectively (Wei et al., 2008). The Dept Experiment also exhibited the presence of 5 quaternary carbon signals at δc 108.6/ C-4, 92.8ppm/C-8, 124.0ppm/C-11, 173.05/C-12, 168.4ppm/C-14 and 127/C-1 respectively (Ilyas et al., 2014). The HMBC and NOESY spectrum correlations between C-1/H-1', H – 1'/C – 1 and H – 1/H – 1' suggested that, the β- glucopyranose moiety was attached at the C – 1 position of the aglycone. The HMQC spectrum has confirmed the corresponding proton signals found as single at δ_H 5.14s for H – 10 and also a singlet for the trisubstituted olefin proton on H – 13 at δ_H 7.56 (s) (Cogne, et al., 2005).

The configuration of C – 6 and C – 7 as an epoxide group was also confirmed from the spectra as a β- configuration (Francis, 2003; Bernstein, 1994). The stereochemistry of C – 8 and C- 10 were ascertained using the NOESY spectrum. The strong NOESY correlations between H – 1/H – 10 also indicated that, the linkage between C – 8 and C – 10 are in α orientation while the H – 10 is in β (Brown, 2003; Kemp, 1991). On the basis of the chemical shift, multiplicity, absolute values for the coupling constant and magnitude for ^1H NMR and ^{13}C NMR spectrum data, indicated the β – configuration at the anomeric position for the glucose moiety. The comparison of spectral data obtained from compound 1 with those of Citrifolinoside were found to be identical. Consequent upon this, compound 1 was also determined as Citrifolinoside, an Iridoid glycoside. Compound **2**: This was obtained as an amorphous brown solid.

The positive FAMS established a molecular formula of $C_{25}H_{28}O_{11}$ which was confirmed by its high resolution positive FAMS as $[M + 1]^+$ 505.132. The UV(MeoH) [max (log ∑) : 203.5 (5.16) 216.8 (5.18), 223.4 (5.16), 243.7 (5.13), 256.2 (5.12), (279.0 (5.30) and 282 nm] while, IR (V: 3392, 1713, 1685, 1628, 1452, 1356, 1278, 1175 and 860cm^{-1}) showed the presence of hydroxyl groups, carbonyl group, carboxyl group, α, β- unsaturated skeleton and an – aromatic ring while values at 216.8, 223 and 282 could be attributed to the iridoid bearing trans cinnamoyl moiety(Hui, et al., 2000). The exhaustive acid hydrolysis of 2 gave glucose and trans – cinnamic acid at ($t_{R\ 5.6min}$):m/z 147(22) $[M-H]^-$73(100) and 45 (35) (Kalpourtzakis et al., 1999). The ^1H NMR spectral data for H – 3 /δ_H 7.4 ppm) indicated the presence of a 4 – substituted enol ether system of an iridoid moiety. The spectrum signal at δ_H 4.6 /H' exhibited the presence of an anomeric proton (Gousiadou, et al., 2007). Signals observed at δ_H 7.1, 6.6, 7.5., 6.8 and 6.3 are all characteristic of an aromatic protons (Dominguez, et al ., 2007). The structure of trans cinnamoyl moiety was established by ^1H NMR data showing two vicinal olefinic protons at (δ_H 7.8 and δ_H 6.7), five aromatic protons at δ_H7.1 (3H) and δ_H 6.8ppm. The Signal at δ_H 7.8/H – 7' and δ_H6.7/ H-8[1] could be attributed to an α and β methylene proton of the trans – cinnamoyl group signifying the presence of vinyl protons (Zhou, et al., 2007). Signals found at δ_H 4.6ppm (^1H, d, 7.8Hz) is a characteristic of a sugar proton which is in conformity with a β – Configuration of an anomeric proton (Neerja, et al., 2008). The glucosyl anomeric proton is linked to the aglycone at position H – 1 as demonstrated by HMBC Spectrum (Zuhal et al., 2007).

In ^{13}C NMR (table 2), peaks are assigned on the basis of chemical shift consideration and comprising with data for glucose. ^{13}C NMR, spectrum was found to exhibit 25 carbon signals, 10 corresponding to aglycone, 9 to trans – cinnamoyl moiety and six attributed to glucose unit. Signal observed at δ_c 127.7ppm, 148.0ppm and 148.00ppm suggested for C-1', C-3' and C-4' are characteristics of aromatic carbons. Signals observed at δ_c 148.0 and 117.1ppm indicate the presence of an -α and β vinylic carbons coupled to δ_c 168.3ppm signifying the presence of a carbonyl group on C – 9' (Yoshiyasu, et al., 2004). The DEPT Experiment exhibited the presence of 7 quaternary carbon signals at δ_c 114.0/C – 4, 75.4/C-5, 136.3/C-8, 127.7/C-1', 146.9/C-3', 149.8/C-4' and 168.3/C-9'.

The $^{1}H - H^{1}$ Cosy, HMQC and HMBC correlation spectra of 1 demonstrate some significant $^{1}H - ^{13}C$ long correlation between H – H and C – 9, C – 1" with H – 1 and H – 3 with H – 1 as clearly shown by HMBC spectrum which further confirmed the attachment position of trans-Cinnamoyloxy group and the glucosyl moiety at C – 8 and C – 7 of the aglycone. The glycosidic linkage observed at δ_H4.6ppm (1H, d) with coupling constant at (J – 7.8H2) is also in conformity with –β –D – glucopyranosyl (Kemp, 2001; Shu-Hua, et al., 2004). The relative stereochemistry of 2 was also determine using NOESY spectrum, which also confirms the H – 1 position of the aglycone as – β – orientation instead of α – orientation. The ^{1}H NMR and ^{13}C NMR spectroscopic data were in great consistent with a C – 8 iridoid monoglucoside moiety (Biswanath, et al., 2009). The comparison of the ^{1}H NMR, ^{13}C NMR data and the coupling constants of Compound 2 with those of Serratoside (Sophon, et al., 2002; Hui et al., 2000) were in great conformity. Thus, on the basis of this discussion, it was resolved that compound 2 was elucidated to be the same as Serratoside.

Compound **3**: This was obtained as an amorphous solid. The UV (MeOH) spectrum exhibited 233.4 and 2.82nm for iridoid moiety and trans cinnamoyl unit. The IR spectrum shows the presence of 3396, 1714, 1689, 1632, 1520 and 1358 values exhibiting the presence of hydroxyl group, carbonyl group an α, β unsaturated skeleton and an aromatic ring while values at 218.4, 225.7 and 283.2 could be attributed to an iridoid and a trans – cinnamoyl, unit (Kemp, 1991). The exhaustive acid hydrolysis of compound 3 gave glucose, rhamnose and trans – cinnamic acid (Yong-Qin et al., 2008). The position of ESI Mass spectrum shows a quasimolecular ion peak at m/z 661 [M + Na]+ suggesting the Molecular formula as $C_{30}H_{38}O_{15}$ which was confirmed by ^{13}C NMR and DEPT spectra. The ^{1}H NMR spectrum (600 MHz, CD_3OD) of compound 3 showed a broad singlet for H – 7 at δ_H 3.62ppm due to small coupling constant existing between H – 7 and H – 6, this hence confirmed C – 8 to be quaternary (Ihsan, C. et al., 2001; Jian-ming, et al., 2003).

A characteristic doublet for an acetal proton H – 1 arisen at δ_H 4.74ppm with a large coupling constant at (J = 9.6H_Z) demonstrated the di hedral angle to be at 180° between the vicinally coupled protons of (H-1 and H – 9). The H – 9/ δ_H 2.72ppm with coupling constant at J = 9.7H_Z was also found to couple with H – 5 and H – 1 of the aglycone (Kalpoulzakis et al., 1999; Kemp, 1991). The coupling of H – 5 exhibited a dihedral angle to be at 0^0, thus revealing the stereochemistry of pyran and cyclopentane ring fused at its position (Gousiadou et al., 2007).

A double doublet signal at δ_H6.42/H-3 with coupling constant (J = 6.0H_Z) suggested a characteristic enol ether signal of an iridoid which was further confirmed by an intense signal in COSY, correlation between H – 3 and H – 4 and also with a weak one on H – 3 and H – 5 (Dominguez et al., 2007). A doublet signal on H – 1' with coupling constant (J = 7.8H_Z) signifies the presence of glucosyl anomeric proton. The long range coupling with H – 1 in HMBC spectrum confirms the C – 1 attachment of the aglycone with H – 1' of the glucosyl moiety. The coupling constant of (J = 7.8Hz) exhibited by H – 1' with δ_H 4.98 shows a dihedral angle of 180°, a di axil relationship between H – 1 and H – 2' vicinal protons of the glucosyl moiety hence confirming the β- configuration of glucose with the aglycone (Zhou et al., 2007, Neerja et al., 2008). The broad singlet signal resonating at δ_H 4.96ppm/H – 1" with a dihedral angle of 60^0 suggest the presence of an anomeric proton, typical of an α – isomer (Zuhal et al., 2005 ; Yoshiyasu , et al., 2004). The signal observed at δ_H 1. 27/H – 6" with J = 6.3 could be attributed to the methyl group corresponding to the rhamnose moiety.

The values obtain at δ_H 4.96/H – 1" and δ_H1.27/H 6" are in great conformity with the α– L – rhamnopyranosyl group (Ilyas et al., 2014; Wei et al., 2008). The location of α – L – rhamnopyranosyl group was determined to be at C – 6 position of the aglycone (catalpol) as observed by the long range connectivity with C -1" of the rhamnopyranosyl moiety (Song, et al., 2006; Kemp, 2001). The COSY, HMBC spectrum has established the linkage of trans – Cinnamoyloxy group of C – 9 being attached to the C – 3 of the rhamnopyranosyl moiety with a glycosidic linkage (IK-Hwi et al., 2001). The structure of trans – cinnamoyl group was also established by the ^{1}H NMR spectrum showing two vicinal olefinic protons at δ_H 7.8 and 6.7 assigned to H -7"' and H – 8"' while signals at δ_H 7.5/H-2"', 7.3/3"', 6.5/H-5"' and 6.4/H-6"' could be attributed to the aromatic protons (Mohammed et al., 2013; Yaching et al., 2004). The DEPT Experiment exhibited the presence of 4 quaternary carbon signals at δc 68.2/C-8, δ C 136.2/-1"', δc150.4/C-4"' and δc 168.4/C=0 respectively. The ^{13}C NMR (table 3), peaks are assigned on the basis of chemical shift, consideration and comparison with data for glucose, rhamnose and catalpol. The spectrum data suggest the presence of 30 carbon signals, 15 attributed to catalpol as aglycone, 6 to the rhamnose group and 9 representing the trans cinnamoyl moiety (Biswanath et al., 2009). The cyclo pentano pyran ring carbons involved in the epoxide ring formation resonate at δ_C 58.7/C-7 and δ_C 68.2/C-8.

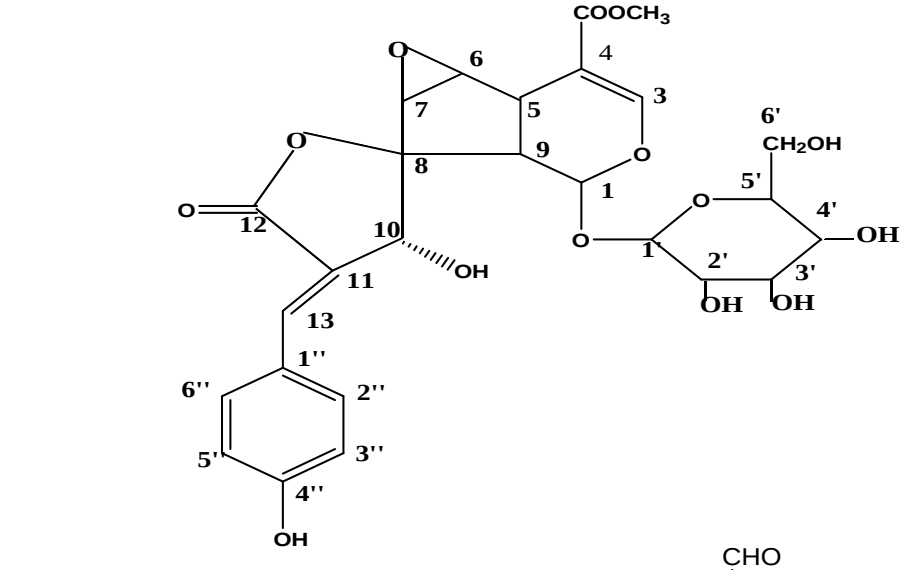
The signal at δ_C 68.2 were found to disappeared in DEPT and as such does not give any connectivity with any proton in HSQC (Neerja et al., 2008). The downfield signal observed at δ_C 94.8/C-1 appeared to be for the acetal carbon signal of the pyran ring as judge by direct attachment of the two oxygen atom (Shengmin et al., 2001). The signal at δ_C 142.3/C-3 was a characteristic of an enol ether carbon (Cogne et al., 2005). The double bond arisen between C – 3 and C- 4 justifies the support for the presence of a cis – double bond (Young and Ling, 2008). The downfield signal observed at δ_C 84.2/C-6 judge the position of rhamnopyranosyl attachment through an ether linkage (Hosny and Rosazza, 1998) to the aglycone moiety. The double doublet of H – 9/ δ_H42.6ppm confirmed the C – 8 position as quaternary hence justifying C– 7 and C – 8 position as an epoxide ring system (Masaki, et al., 2001; Kemp, 1991). The HMBC correlation observed between acetate bearing methylene carbon at δ_C 63.02/C-10 and proton signal at δ_H 3.62 and 2.72 corresponding to C-7 and C-9 enabled us to decide the position of C – 8 (Bernstein, 1994).

The exhaustive acid hydrolysis of compound 3 afforded glucose and rhamnose to be – β – D – glucopyranose and - α

– L rhamnopyranose and trans – cinnamic acid as identified by comparing with authentic samples in TLC.

The comparative studies of the spectral data obtained from (HMBC, HSQC, DEPT and NOESY) and comparison of aglycone with (catalpol) thus, it was resolved that compound 3 was elucidated to be determined as 6 – O – (3" – O

– trans – cinnamoyl) - α – L – rhamnopyranosyl catapol heptaacetate.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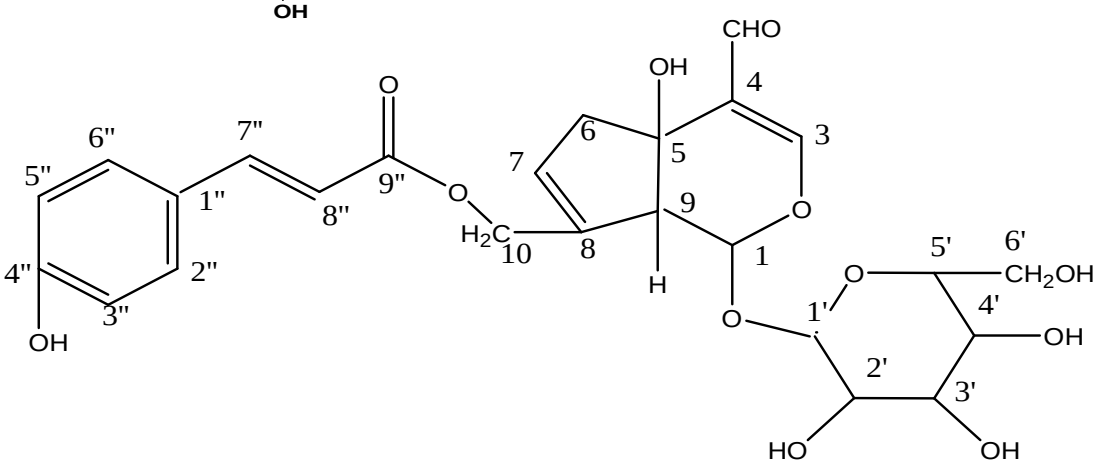
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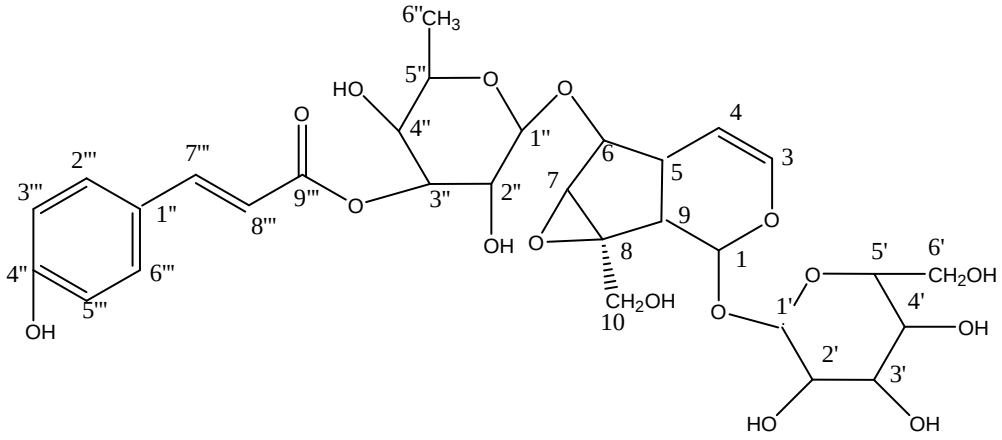
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Table 1: ^{1}H NMR (600 MH_z) and ^{13}C NMR (150MH_z) Spectral data for Compound 1in (CD_3 OD; δ in ppm, J in Hz)

Position	δ_H	δ_C
1	5.32 d	93.2
3	7.41 d(2.0)	154.0
4	-	108.6
5	3.4 dd (2.0, 8.4)	34.5
6	4.03 d (2.5)	58.4
7	3.9 d (2.5)	58.4
8	-	92.8
9	2.46 d (8.7)	46.4
10	5.14 s	70.2
11	-	124.0
12	-	173.05
13	7.56 s	144.04
14	-	168,4
OCH_3	3.74 d(8.0)	51.7
1'	4.43 m	99.4
2'	3.07 m	74.5
3'	3.26 m	78.4
4'	3.26 m	71.2
5'	3.22 m	77.4
6'	3.74 m	62.5
	3.62 m	
1"	-	127.5
2"	7.6 d(8.2)	134.6
3"	6.84 d(8.4)	117.08
4"	-	162.5
5"	6.87 (8.4)	117.04
6"	7.68 (8.4)	134.68

Table 2: ^{1}H NMR (600MH$_Z$) and ^{13}C NMR (150M H$_Z$) Spectral Data for Compound 2 (CD$_3$OD; δ in ppm, J in H$_Z$)

Position	δ_H	δ_C
1	5.8 (1H, d, J = 1.1 H$_Z$)	92.4
3	7.4 (1H, S)	151.2
4	-	114.0
5	-	75.4
6	1.9)1H, M, J = 2.73)	38.0
7	5.6 (brs)	129.3
8	-	136.3
9	3.59 (d)	57.4
10	(a) 5.11 (d) J = 13.6 (b) 5.22 (d) J = 13.8	62.1
1'	9.3 (s)	188.2
1'	-	127.7
2'	7.1	115.2
3'	-	146.9
4'	-	150.8
5'	6.6	114.7
6'	6.8	123.2
7'	7.8	148.0
8'	6.7	117.1
9'	-	168.3
1"	4.6 (1H, d, J = 7.8HZ)	99.6
2"	3.2 (3H, M)	73.0
3"	3.3 (3H, M)	76.4
4"	3.4 (3H, M)	71.0
5"	3.4 (3H, M)	78.2
6"	3.6 (1H dd 6.0)	62.4

Table 3: ^{1}H NMR (600MH$_Z$) and ^{13}C NMR (150 MH$_Z$) Spectral Data for Compound 3 (CD_3OD; δ in ppm, J in Hz).

POSITION	δ_H	COSY	δ_C
1	4.74(1H, d J = 9.6)	H - 9	94.2
3	6.42 (1H, dd, J = 6)	H – 4, H - 5	142.3
4	5.11(1H, dd J=6, 4.6)	H-3, H-5, H-6	103.6
5	2.61(1H, M)	H-3, H-4, H-6	36.1
6	3.84 (1H, dd J =8.6)	H-1", H-5, H-7	84.2
7	3.62 (1H, brs)	H - 6	58.7
8	-	-	68.2
9	2.72(1H, dd J=9.7)	H -1	42.6
10a	3.90 (1H, d, J=12.6)	H -10b	63.02
10b	4.76 (1H, d, J=12.6)	H – 10a	
Glycosyl			
1'	4.98 (1H, d, J=7.8)	H - 2'	98.6
2'	5.23 (1H, dd, J=9.2)	H - 1'	73.4
3'	4.94 (1H, t, J=9.5)	H 4'	71.2
4'	5.23 (1H, t, J=9.2)	H – 3', H - 5'	68.7
5'	3.63 (1H, M)	H-6'a,b H-4	72.6
6'a	4.15 (1H, dd, j=12.3)	H-5', H-6b	62.4
6'b	4.34 (1H, dd, J=12.4)	H-5', H -6a	
Rhamnosyl			
1"	4.96 (1H, brs)	H – 2"	103.4
2"	5.34 (1H, dd, J=3.5)	H – 1", H.3"	70.9
3"	5.66 (1H, dd, J=9.6)	H-2", H-4"	69.3
4"	5.41 (1H, t, J=9.8)	H-3", H-5"	72.0
5"	4.09 (1H, dd, J=6.2)	H-4", H-6"	62.5
6"	1.27 (3H, d, J=6.3)	H-5"	17.9
Cinnamoyl			
1"'	-		136.2
2"'	7.45(d)		129.1
3"'	7.32 (d)		128.4
4"'	-		150.4
5"'	6.5 (d)		129.2
6"'	6.4 (d)		129.6
7"'	7.8		118.6
8"'	6.7 (d)		146.8
C = 0	-		168.4

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