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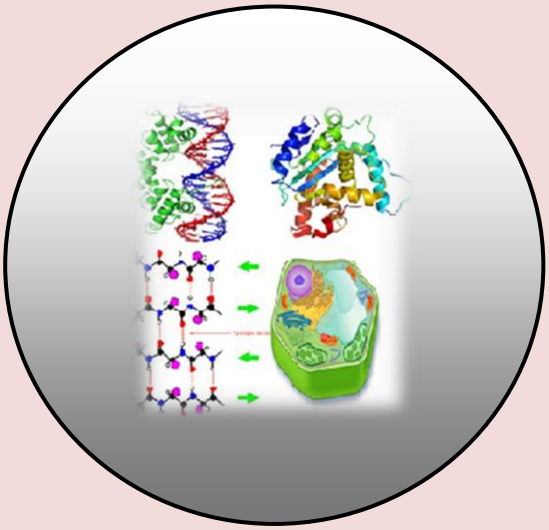

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RESEARCH PAPER

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Isolation and Structure Elucidation of a Novel Pregnane Pentaglycoside Latoside from *Dregea lanceolata*

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ABSTRACT

A new pregnane glycoside designated as Latoside was isolated from chloroform soluble extract of Dregea lanceolata (family: Asclepiadaceae). The novel structure of pregnane glycoside was elucidated to be as 12-O-acetyl preg-5-ene, 3β,8β,14β,20-tetrol-3-O-β-D-cymaropyranosyl-(1→4)-O-β-D-cymaropyranosyl-(1→4)-O-β-D-cymaropyranosyl-(1→4)O-β-D-mannopyranosyl-(1→4)-β-D-glucopyranoside on the basis of results obtain from chemical transformation/degradation, ^{1}H, ^{13}C and 2D NMR experiments as well as Mass specrometery.

Keywords: Latoside, Dregea lanceolata, pregnane glycoside, Asclepiadaceae, NMR and Mass spectrometery.

INTRODUCTION

Plants belonging to family Asclepiadaceae are reported to be a rich source of steroids (pregnane and cardenolides) (Hosseini et al., 2019, Zhang et al., 2022, Yan et al., 2022, Sireesha et al., 2022), terpenes and their glycosides (Deepak et al., 1997, Kumar et al., 2019, Wei et al., 2020). Large numbers of pregnane glycosides have been isolated from various species of Asclepiadaceae family (Deepak et al., 1989, Khare et al., 1986, Deepak et al., 1997, Kumar et al., 2019). These pregnane glycosides have been reported to have antitumor, anticancer (Hussain et al., 2015), antioxidant, antidyslipidemic activities (Sethi et al., 2008), anti-inflammatory, antimicrobial (Babu et al., 2008) and anti-complementary (Piacente et al., 1998) activities. *Dregea lanceolata* is a sub erect climbing shrub belonging to family Asclepiadaceae. It is commonly grown in some districts of Maharashtra state (Garad et al., 2015).

Previous investigations on *Dregea lanceolata* have led to the isolation of pregnane glycosides such as dregealin (Krishna et al., 1990), drelin, ceolin (Krishna et al., 1991), and lancoside A (Kumar et al., 2019). Structurally, pregnane glycosides are comprise of steroidal moiety having a basic perhydrocyclopentanophenanthrene moiety with OH group substituted at different positions of steroid and sugar (mostly 2, 6-dideoxy sugars and normal sugars) unit at position C-3. In this paper we describe the isolation and structural elucidation of novel pregnane oligoglycoside, Latoside (1) from chloroform soluble extract of *Dregea lanceolata*. Compound Latoside 1 was designated as 'L'.

MATERIAL AND METHODS

General Experimental Procedures

1D NMR and 2D NMR experiments were performed on AVANCE 400 MHz Bruker spectrometer in $CDCl_3$.TMS was used as internal standard. The ES-MS spectrum was measured on AEI-MS-30 mass spectrometer. Melting point was recorded on Buchi melting point B-540 apparatus and is uncorrected. Optical rotation was measured with an automatic polarimeter AA-5 series of optical activity. Column chromatography was performed over silica gel 60-120 mesh (BDH). TLC was performed on silica gel-G (BDH), compound was detected by spraying with 50% aq. H_2SO_4 solution followed by heating.

Plant Extraction

Shade dried, powdered plant of *D. lanceolata* (10 kg) were extracted by the method employed for pregnane glycosides [Neupane et al., 2017] using 50-95% ethanol. Chloroform extract (2.0 g) was fractionated by repeated column chromatography over Silica gel using solvent mixture of C_6H_6-EtOAC and $CHCl_3$-MeOH as eluents afforded Latoside (38 mg).

Mild acid Hydrolysis of Latoside (1)

To a solution of 1 (15 mg) in 80% aq. 1,4-dioxane (1ml) was added 0.05 N H_2SO_4 (1ml) and the solution was left at room temp using the method of Rangaswami and Reichstein (Rangaswami and Reichstein, 1949). The dioxane was removed under reduced pressure, the aq. portion was repeatedly extracted with $CHCl_3$ and the organic layer was washed with water, 2N Na_2CO_3 again with water and dried over Na_2SO_4 and evaporated to afford genin 4 (3mg) m.p. 183-185^{0}C $[\alpha]_D$-52.5^o which was later identified as 12-O-acetyl-lineolon. The water layer was dried under vacuum and was purified by column chromatography which afforded three chromatographically pure sugars which were identified as cymarose, mannose and glucose (TLC, PC, $[\alpha]_D$).

Methanolysis of (4) by Zemplen Method (Zemplen and Kiss, 1927)

To a solution of 1 (2.5 mg) in absolute MeOH (0.5 ml) was added sodium methoxide (0.05 ml) and the mixture was kept at room temp. When the reaction was complete (TLC), it was neutralized with IR 120 H resin and filtered. MeOH was removed under reduced pressure yielding the product **11** (1.2 mg).

Acetylation of Latoside

Compound 1 (15g) was acetylated with pyridine (2ml) and acetic anhydride (2ml) at 60^O C and the mixture was kept overnight, yielding nonaacetate **2** (14.8 g).^{1}H NMR- δ2.08-2.17 (9xOAc), 3.19-3.21 m (H-4 of S_1, S_2, S_3), 3.26 m (H-4 of S_4) 3.52 m (H-3 of aglycon).

Latoside

Colorless crystals, mp 130-132ºC, $[\alpha]_D^{25}$= +28º.It gave positive Liebermann Burchardt test, Xanthydrol test, Keller Killiani test, Partridge test and tetra-nitro methane test.

^{1}H NMR (400MHz, $CDCl_3$): δ 5.48 (1H, m, 6-H), 5.35 (1H, t, H-12) 4.84 (1H, dd, J= 8.0, 2.6 Hz, H-1 in S_1), 4.80 (1H, dd, J= 8.0, 2.0Hz, H-1 in S_4), 4.78 (1H, dd, J= 8, 2Hz, H-1 in S_2), 4.59 (1H, d, J= 8.0 Hz, H-1 in S_5), 4.48 (1H, dd, J= 8.0, 2.0 Hz, H-1 in S_3), 3.66 (3H, s, S_1-OCH_3), 3.44 (3H, s, S_2-OCH_3), 3.38 (3H, s, S_3-OCH_3), 3.52 (1H,m, H-3 in aglycon), 2.16 (3H, s, 21-CH_3CO), 1.95 (3H,s, 12-OAc),1.6(2H, 11-CH_2) 1.32-1.25 (3H, d, sec cym-CH_3), 1.12 (3H, s, 10-CH_3), 1.20 (3H, s, 13-CH_3).

^{13}C NMR (100MHz, $CDCl_3$): δ38.9(C-l), 29.2 (C-2), 78.2 (C-3), 38.0 (C-4), 139.6 **(**C-5), 117.6 (C-6), 35.5 (C-7). 73.3 (C-8), 43.3 (C-9), 39.3 (C-l0), 22.4 (C-ll), 68.3 (C-12), 53.9 (C-13), 83.8 (C-14), 35.7 (C-15), 25.1 (C-16-), 55.2 (C-17), 11.5 (C-18), 17.8 (C-19), 68.5 (C-20), 22.7 (C-21). 95.92(C-1_{S1}), 33.10 (C-2_{S1}), 77.30 (C-3_{S1}), 72.60 (C-4_{S1}), 68.50 (C-5_{S1}), 18.2(C-6 $_{S1}$), 58.1 ($OCH3_{S1}$), 99.16 (C-1_{S2}), 35.50 (C-2_{S2}), 77.0 (C-3_{S2}), 72.4 (C-4_{S2}), 68.50 (C-5_{S2}), 18.4 (C-6 $_{S2}$), 58.0 ($OCH3_{S2}$), 99.64 (C-1_{S3}), 35.7 (C-2_{S3}), 76.6 (C-3_{S3}), 72.40 (C-4_{S3}), 71.7 (C-5_{S3}), 18.4 (C-6 $_{S3}$), 58.0 ($OCH3_{S3}$), 101.15 (C-1_{S4}), 72.8 (C-2_{S4}), 75.1 (C-3_{S4}), 68.50 (C-4_{S4}), 77.30 (C-5_{S4}), 61.9 (C-6 $_{S4}$), 101.15 (C-1_{S5}), 72.5 (C-2_{S5}), 72.8 (C-3_{S5}), 71.4 (C-4_{S5}), 72.30 (C-5_{S5}), 61.9 (C-6 $_{S5}$).

ES-MS: m/z 1203 $[M+K]^+$, 1164 $[M]^+$,, 1002, 840, 757, 739, 725, 721, 705, 696, 693, 675, 657, 552, 408, 393, 390, 363, 324, 270, 262, 253, 245, 210, 138, 120, 105.

RESULTS AND DISCUSSION

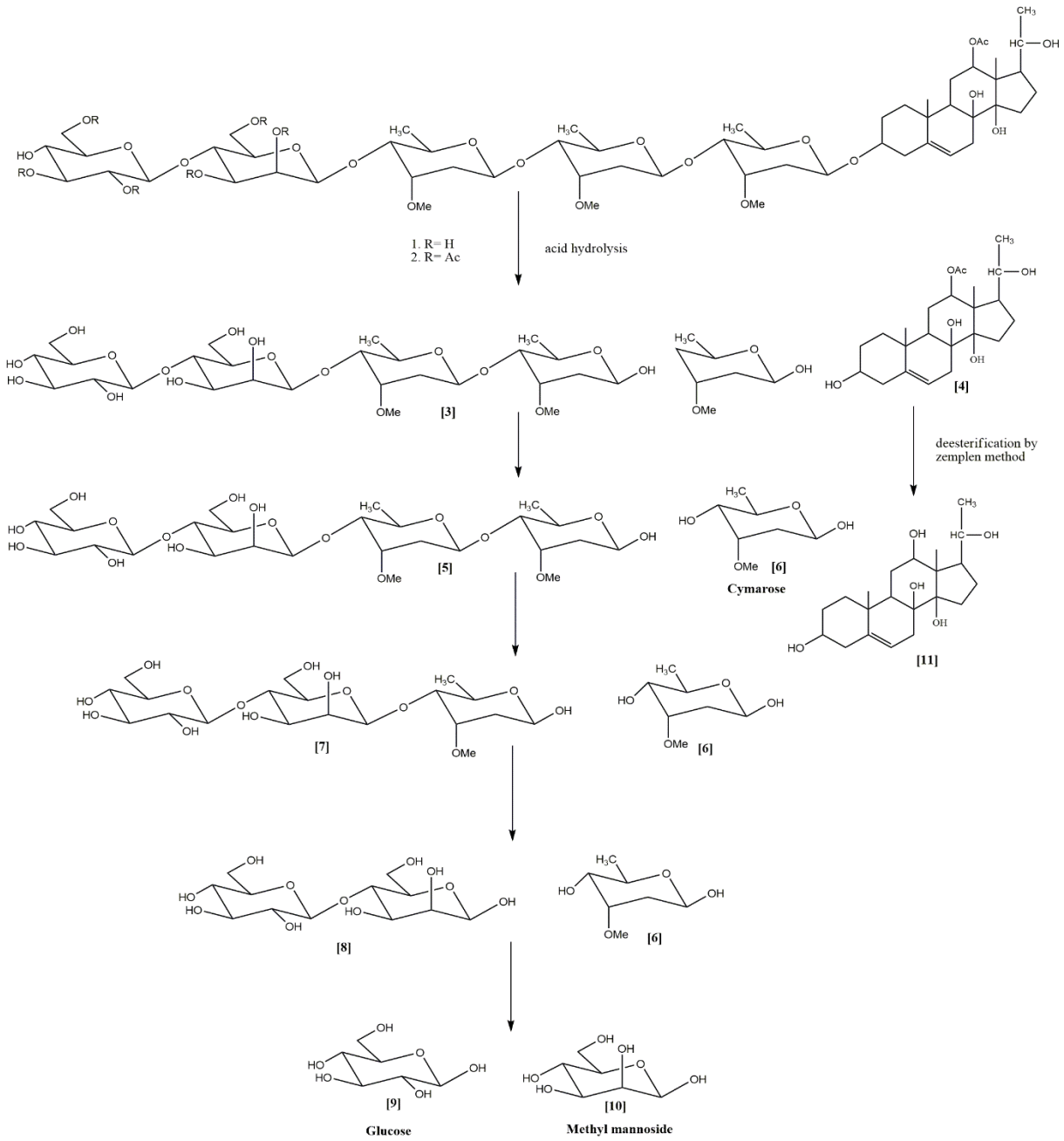
Chloroform soluble extract of *Dregea lanceolata* was fractionated on a silica gel afforded compound1.

Latoside (1)

Latoside (1), mp = 130-132^{o}C, $[\alpha]_D^{25}$= +28^o, $C_{56}H_{92}O_{25}$, m/z 1164$[M]^+$ gave positive Liebermann Burchardt test(Abisch et al., 1960),Xanthydrol test (Tschesche et al., 1953), Keller Killiani test (Nagata et al., 1957),Partridge test (patridge et al., 1949), and tetra-nitro methane test (Ostroisslensley et a.,l 1910)indicating it to be steroidal glycoside of 2,6-dideoxy hexose(s) and normal sugar with olefinic bond. It also gave alkaline hydrolysis indicated the presence of ester function in the molecule.

The ^{1}H NMR spectrum of 1 showed five anomeric proton signals at δ 4.84, 4.80, 4.78, 4.59 and 4.48 of one proton each suggesting that it to be a pentaglycoside. The pentaglycoside nature of 1 was further ascertained by the presence of five anomeric carbon signal at δ 101.15 (2C), 99.64, 99.16 and 95.92 in its proton noise decoupled ^{13}C NMR spectrum. The HSQC spectrum of **1** showed five crossed peak of carbon and hydrogen (^{13}C x ^{1}H) in anomeric region at 101.15 x 4.48, 101.15 x 4.59, 99.64 x 4.78, 99.16 x 4.8 and 95.8 x 4.84 supported that **1** was a pentaglycoside.

The identification of sugars, genin and their sequence in 1 was identified by very mild acid hydrolysis by 0.005 N H_2SO_4. For convenience the sugars present were designated as S_1, S_2, S_3, S_4 and S_5. After five days two new spots (**3** & **4**) were appeared, out of which one polar spot **3** could not be identified and the compound with faster mobility was identified as aglycon **4,** however the polar spot **3** could be expected as pentasaccharide whereas component with faster mobility was identified as genin **4** mp 183–185^{o}c, $[\alpha]_D$ 52.5^o. After seven days two new spots **5** and **6** were appeared out of which **6** was having the comparable mobility with the authentic sample of cymarose (Krassoetal., 1963) supporting that the cymarose [2, 6-dideoxy-3-O-methyl-D-ribohexose] was first sugar from the reducing end, while the other new spot may be of the Tetrasaccharide **5**.



Scheme 1. Acid hydrolysis of Latoside (1).

After ten days one more spot **7** was appeared which was having the faster mobility than the compound **5** supporting the fact that **7** may be a trisaccharide and the sugar broken from the reducing end of **5** may be the cymarose **6.** After twelve days one more spot was developed on the TLC plate which was faster in mobility with the trisaccharide **7** which was developed by partridge reagent may be the disaccharide **8**. The disaccharide was converted to its methylglycoside which on acid hydrolysis gave D-glucose (Bohem et al., 2003) and methyl mannoside (**9** and **10**) (TLC, PC). These results showed that glucose was on non-reducing end and mannose was next sugar to cymarose involved in glycosidic linkage also confirmed the sequence of the sugar and aglycon in molecule as Glu-Man-Cym-Cym-Cym-aglycon from the non-reducing end (Scheme 1).

Table 1. ^{13}C NMR data of Latoside (1).

Carbon	Chemical shifts of Latoside (δ)	Carbon	Chemical shifts of Latoside(δ)
1	38.9	D-Cym (S-2)	
2	29.2	1	99.16
3	78.2	2	35.5
4	38.0	3	77.0
5	139.6	4	72.4
6	117.6	5	68.5
7	35.5	6	18.4
8	73.3	OMe	58.0
9	43.3	D-Cym (S-3)	
10	39.3	1	99.64
11	22.4	2	35.7
12	68.3	3	76.6
13	53.9	4	72.4
14	83.8	5	71.7
15	35.7	6	18.4
16	25.1	OMe	58.0
17	55.2	D-Mann (S-4)	
18	11.5	1	101.15
19	17.8	2	72.8
20	68.5	3	75.1
21	22.7	4	68.5
D-Cym (S-1)		5	77.3
1	95.92	6	61.9
2	33.10	D-Glc (S-5)	
3	77.30	1	101.15
4	72.6	2	72.5
5	68.50	3	72.8
6	18.2	4	71.4
OMe	99.16	5	72.3
		6	61.9

The genin **4**, $C_{23}H_{36}O_6$, mp 183–185^{o}c, $[\alpha]_D$ 52.5^{o} on desterification by Zemplen method gave genin **11** (Zemplen et al. 1926) **,** $C_{21}H_{34}O_5$m.p. 161^{0}C have the comparable mobility with authentic sample of preg-5-ene 12-O-acetyl 3,8,14,20 tetrol, which was obtained from reduction of aglycon obtained from acid hydrolysis of earlier isolated compound Lancoside A (Kumar et al., 2019) confirming that genin was esterified preg-5 ene 3,8,12,14,20 pentol. The structure of genin **4** was further confirmed by the ^{1}H NMR of compound **1** at 400 MHz which showed three singlet of three protons each at δ 1.95, 1.12 and 1.20, a multiplet of H-20 at δ4.21 along with H-12 methine proton triplet at δ5.35 and a multiplet at δ5.48 for H-6 proton of aglycon confirmed that genin was 12-O-acetyl preg-5 ene 3, 8, 14, 20 tetrol.

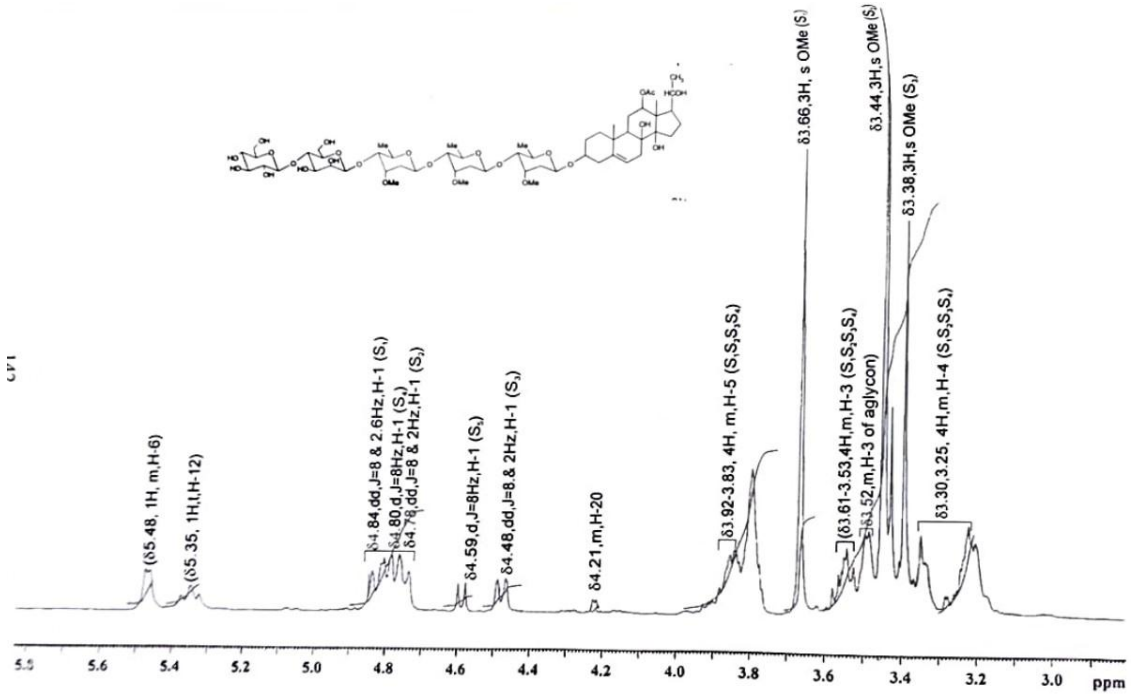


Figure 1. 400 MHz ^{1}H NMR Spectrum of Latoside (1).

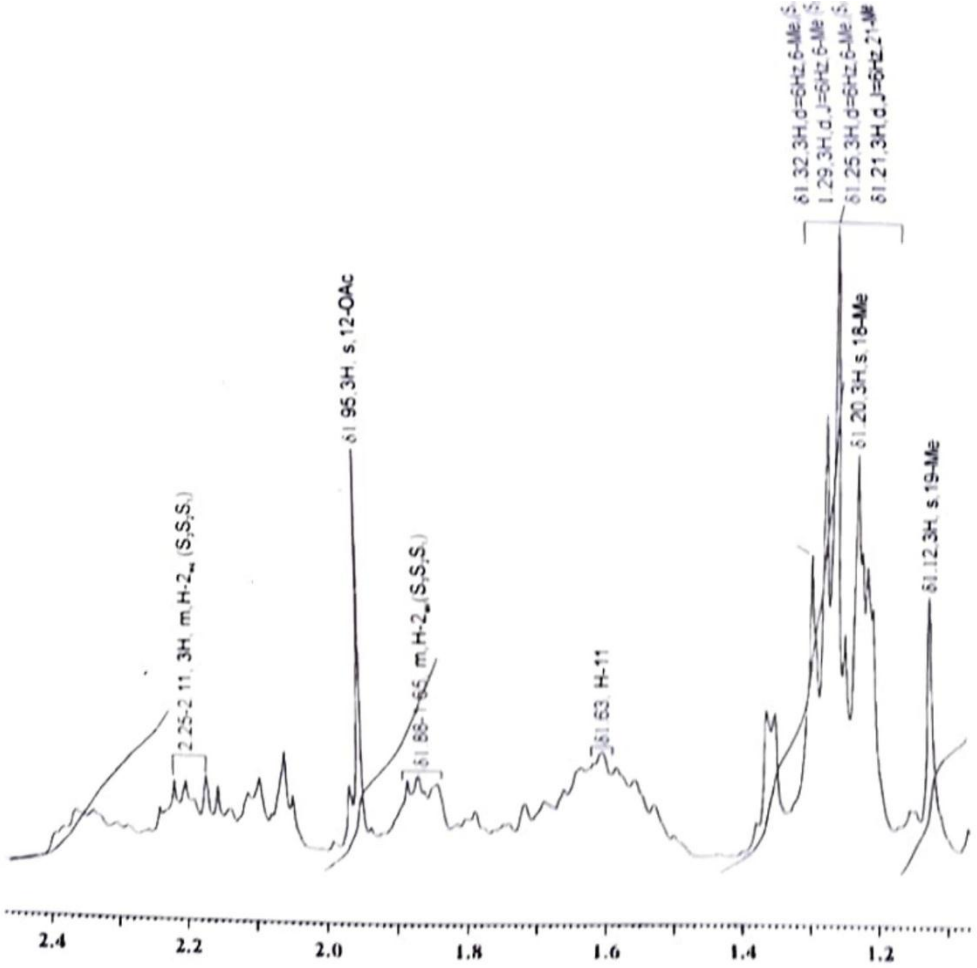


Figure 2. 400 MHz ^{1}H NMR Spectrum of Latoside (1).

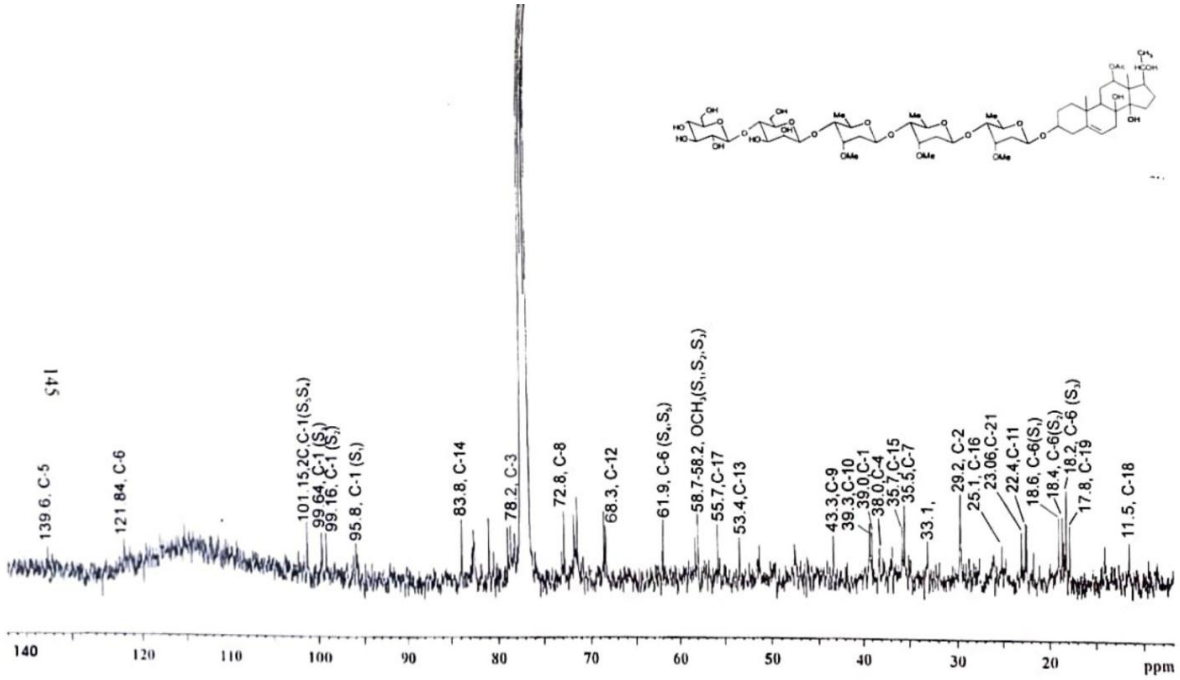


Figure 3. 400 MHz ^{13}C NMR Spectrum of Latoside (1).

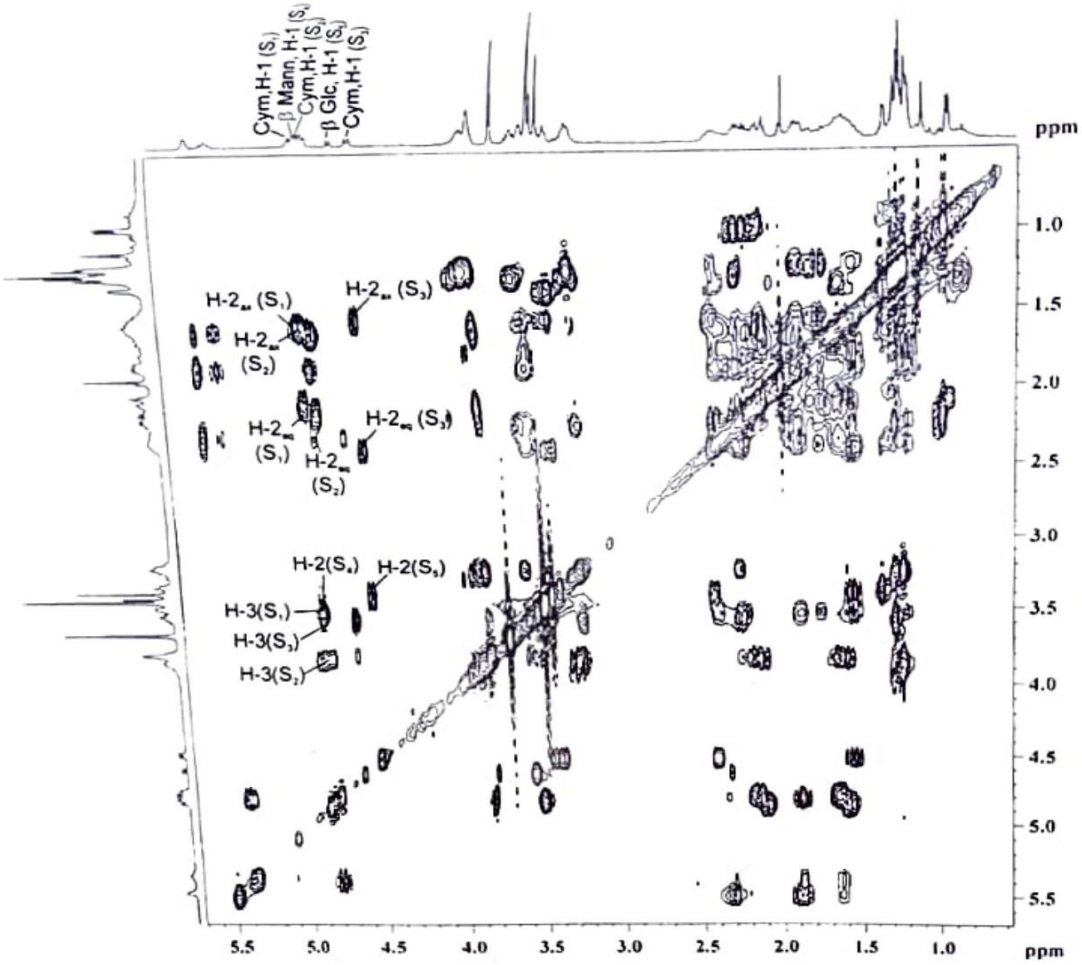


Figure 4. ^{1}H-^{1}H TOCSY Spectrum of compound Latoside (1) in $CDCl_3$.

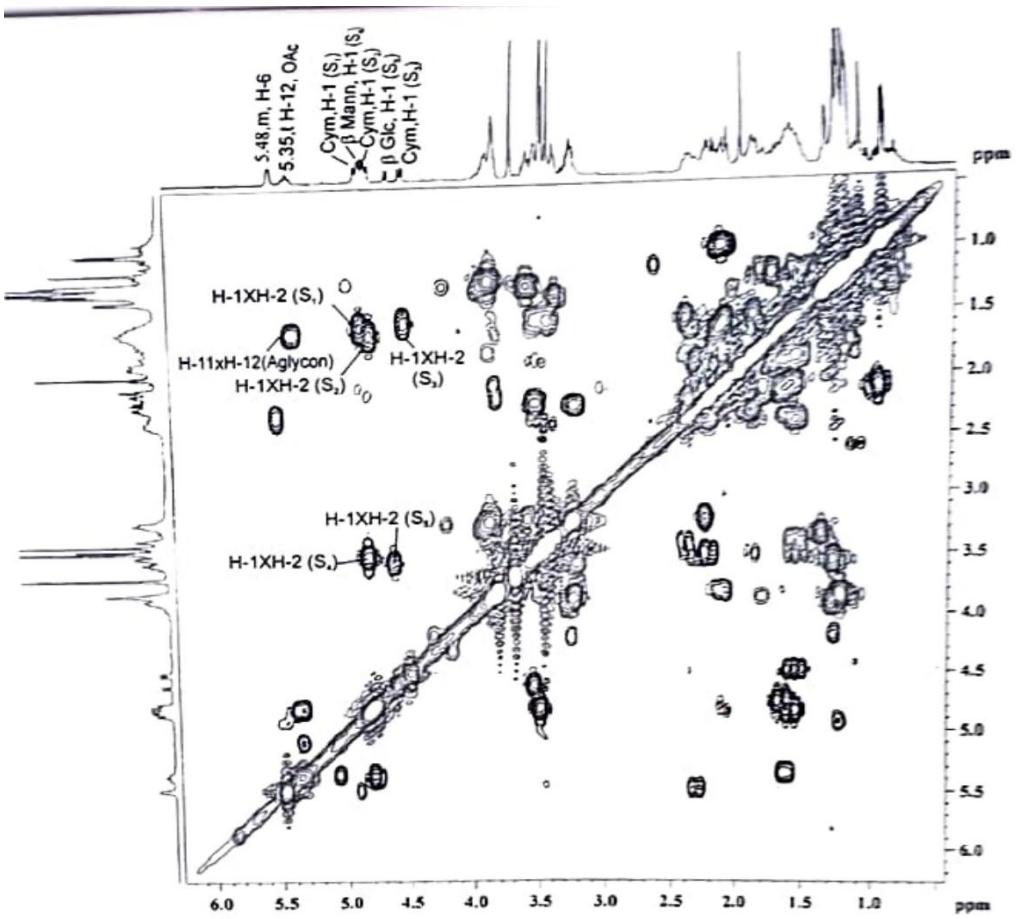


Figure 5. ^{1}H-^{1}H COSY Spectrum of compound Latoside (1) in $CDCl_3$.

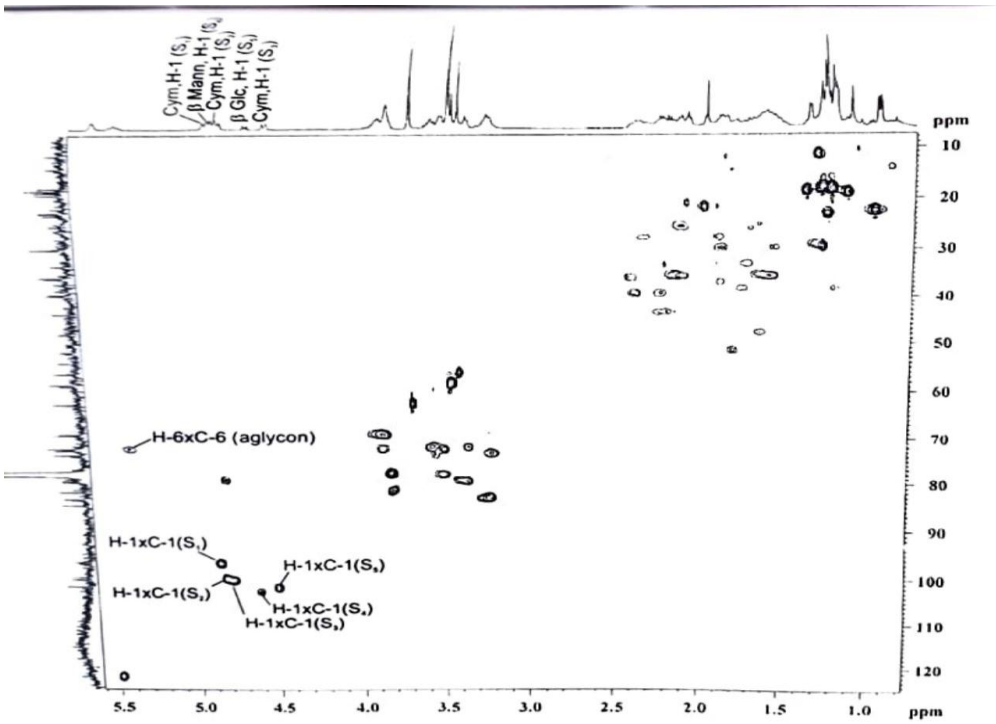
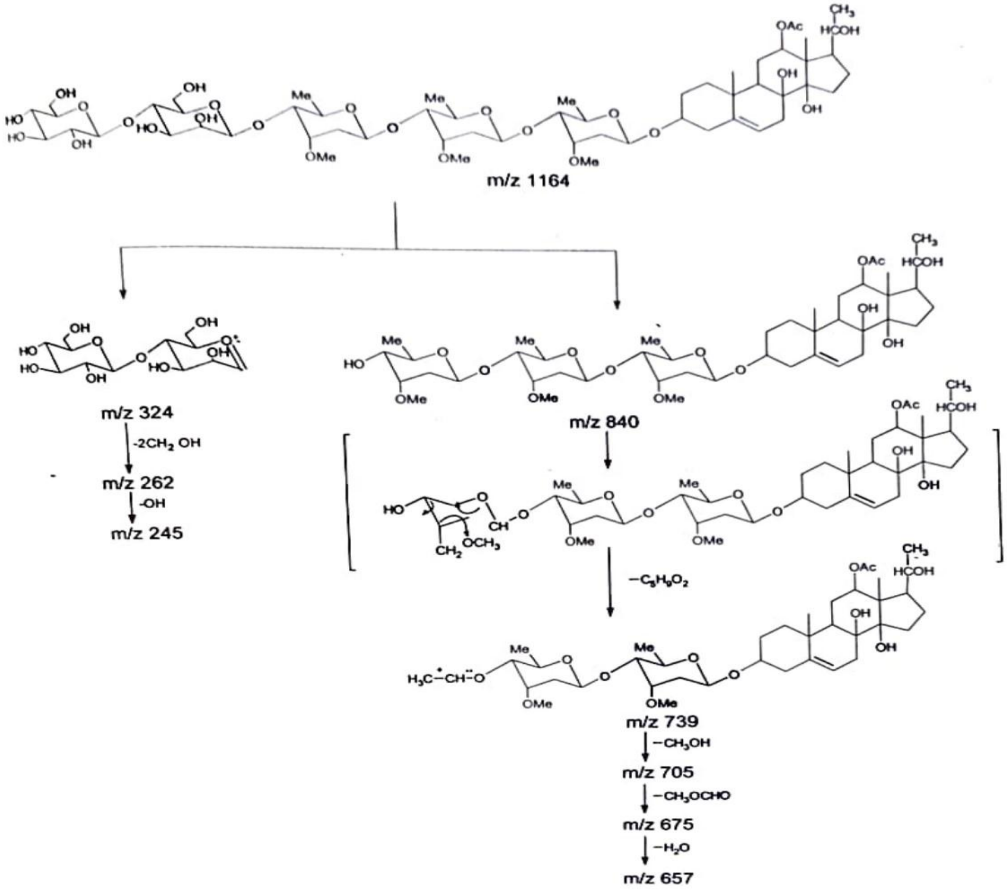
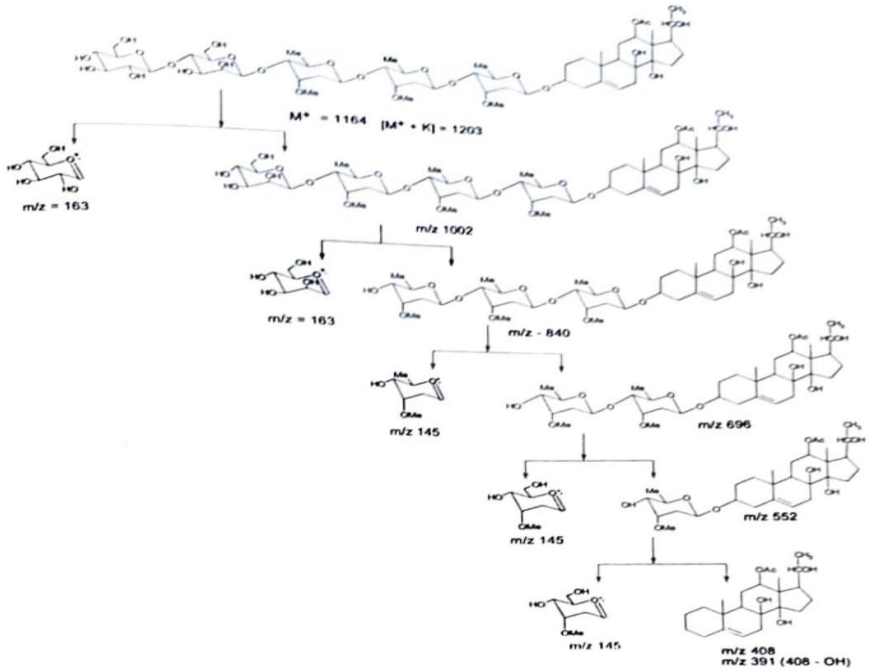


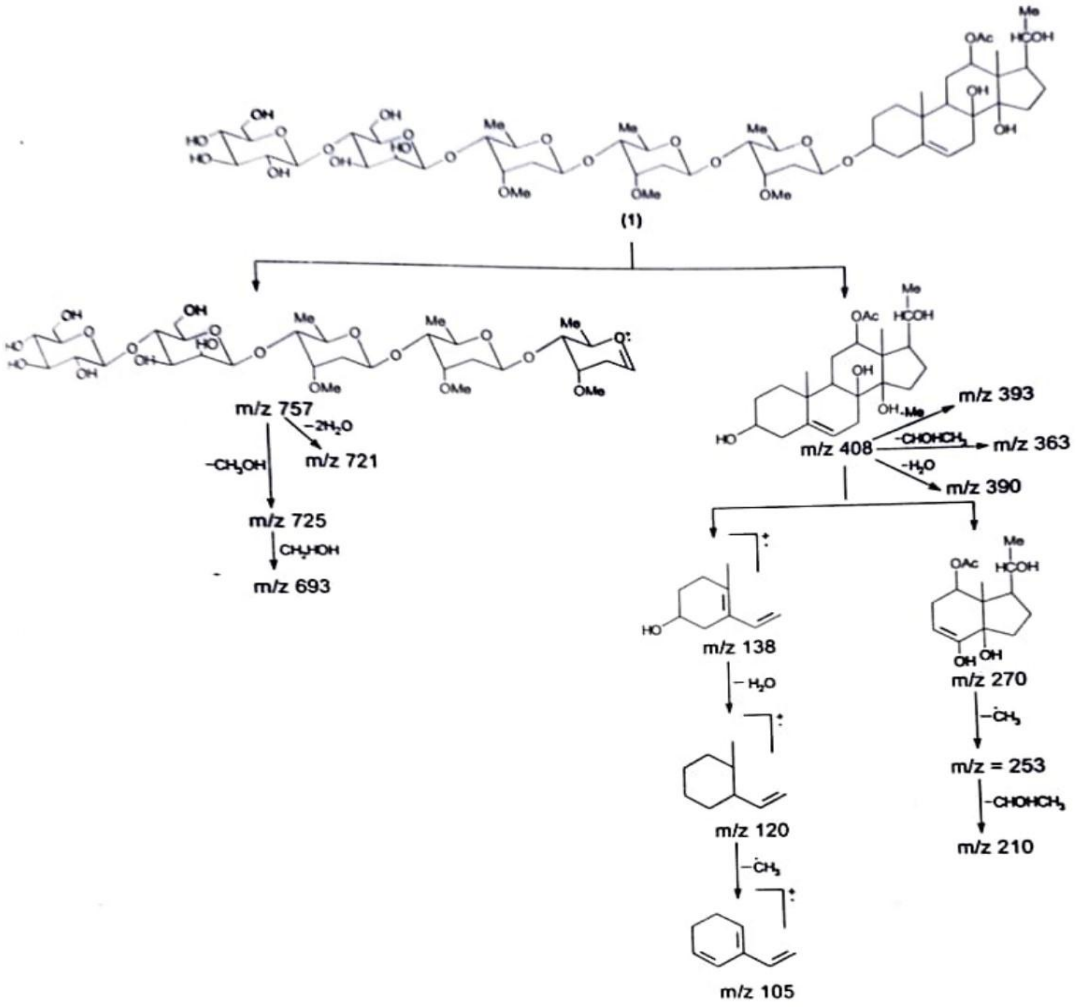
Figure 6. HSQC Spectrum of Latoside (1) in $CDCl_3$.



Scheme 1. Mass fragmentation of Latoside (1).



Scheme 2. Mass fragmentation of Latoside (1) by repeated H^+ transfer.

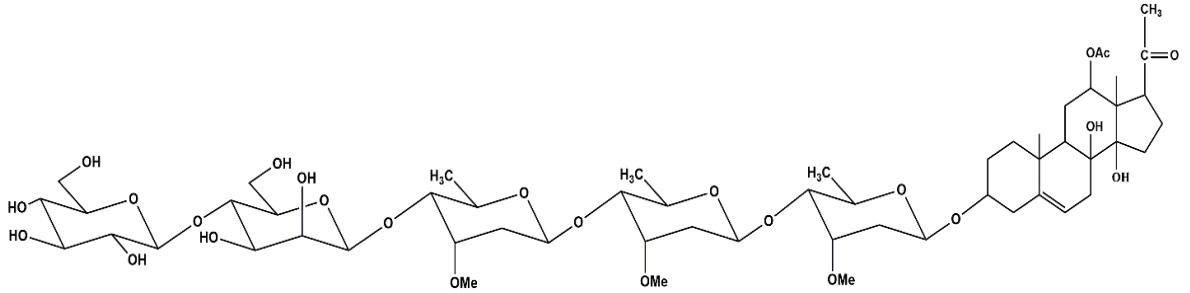


Scheme 3. Mass fragmentation of Latoside (1).

The ^{1}H NMR spectrum of the glycoside **1** showed the configuration of glycosidic linkages. The configuration of five anomeric protons in the five sugar unit could be easily derived from the value of their coupling constants of their signals at δ 4.84 (1H) as double doublet (J = 8 and 2.6 Hz) of S_1, 4.78 (1H) as double doublet (J = 8 Hz and 2 Hz) of S_2, 4.48 as double doublet (J = 8 and 2 Hz) of S_3, 4.80 (1H) as doublet (J = 8 Hz) of S_4 and 4.59 (1H) as doublet with J = 8 Hz of S_5. The larger value of coupling constant of three 2, 6-dideoxy sugars S_1, S_2, S_3 were typical of axial configuration of hexopyranose in $^{4}C_1$ (D) conformation indicating β glycosidic linkage in all three cymarose units while the two normal sugars present in pentaglycoside which gave their anomeric signals at δ 4.80 (J = 8 Hz) as doublet and 4.59 as doublet (J = 8) showed that S_4 (mannose) and S_5 (glucose) also showed β glycosidic linkage.
The ^{1}H NMR also contained a multiplet of one proton at δ 5.48 which was assigned to the vinylic proton present at C-6 of the aglycon moiety. The singlet of three protons at δ1.95 along with a downfield shifted methine proton triplet at 5.35 was due to acetyl group present at H-12 of aglycon confirming that acetyl group was present at H-12 of aglycon which was substantiated by a cross peak at δ 5.35x1.6 in ^{1}H-^{1}H COSY spectrum showed the presence of methylene group at C-11 of the aglycon.

^{1}H NMR also contained a multiplet of H-20 at δ4.21, a multipet present at δ3.52 was due to H-3 proton of aglycon which also has cross peaks in the methylene region in the COSY spectrum. The two singlets of three protons each at δ1.12 and 1.20 were assigned for two angular methyl groups present at C-10 and C-13. The presence of three methoxy groups of sugars S_1, S_2 and S_3 present in **1**, were confirmed by the presence of three singlets of three protons each at δ3.66, δ3.44 and δ3.38 in its ^{1}H NMR spectrum. The doublets of secondary methyl of cymarose gave the ^{1}H signal in the reigon δ1.32-1.25 as double doublets. The ring methine protons of S_1, S_2, S_3, S_4 and S_5 were assigned on the basis of TOCSY experiment by comparison with the reported values in literature. The above assignments ofthe sugar ring methine protons were also supported and confirmed by COSY, HSQC and TOCSY experiments of 2 D NMR spectroscopy of **1**.The remaining characteristic carbon signals of **1** were also supported the derived structure which were shown in Table 1. To confirm the glycosidic linkages of **1**, it was acetylated with Ac_2O in pyridine at 100^0C which afforded an amorphous nona-O-acetyl derivative **2**. The structure of nona acetate was finally elucidated by the analysis of its ^{1}H NMR. It showed the presence of nine singlets of three protons each in the region δ2.17-2.083 which accounted for the seven hydroxy groups present in five sugar units, one acetate which was present at position **12** of aglycon moiety and one hydroxy group which was present at position C-20 of aglycon moiety. The chemical shifts of H-3 methine proton of the aglycon remain unchanged in the ^{1}H NMR spectra of compound **1** and its acetylated derivative **2**indicated that it was involved in glycosidic linkage with cymarose. The absence of any downfield shift in the H-4 methine proton of cymarose upon acetylation at δ3.19 to 3.21 for S_1, S_2, S_3 sugars respectively showed that C-4 of all cymarose units were involved in glycosidic linkages. Further the C-4 of S_4 (mannose) ^{1}H NMR of **1** did not show any downfield shift, also confirmed that C-4 of mannose was involved in glycosidic linkage by (1→4) with the S_5 (glucose). The ES mass spectrum of **1** displayed highest mass ion peaks at m/z 1203 and m/z 1164 which were due to $[M+K]^+$ and $[M]^+$ respectively. The sugar sequence was confirmed via the fragmentation path which showed ion at m/z 1002[1164-S5], 840[1002-S4], 696[840-S3], 552[696-S2] and 408[552-S1].The mass peak at m/z 408 corresponded to genin which further fragmented to give other respective fragment peak at m/z 393 (408 - Me), m/z 363 (408 – $CHCOCH_3$), m/z 347 (365– H_2O), m/z 390 (408-H_2O), m/z 330 (390-AcOH), m/z 225 (270-$CHOHCH_3$), m/z 209 (227-H_2O) were supported the presence of two methyl, one acyl and three hydroxyl group in genin (Bosso et al., 1978).

In the light of foregoing evidence, the structure of novel substance 1was established as **12-O-acetyl preg-5-ene, 3,8,14,20 tetrol 3-O-β-D-cymaropyranosyl (1→4)-O-β-D-cymaropyranosyl (1→4)-O-β-D-cymaropyranosyl (1→4)-O-β-D-mannopyranosyl (1→4) -β-D-glucopyranoside.**



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