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Pregnanes and pregnane glycosides from *Marsdenia roylei*

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Abstract

Two pregnanes namely desacylkondurangogenin C (**1**) and deniagenin (**3**, new) and two new pregnane glycosides designated as denin (**5**) and marsin (**12**) have been isolated from chloroform soluble extract of dried stem of *Marsdenia roylei*. Chemical and spectroscopic evidences are consistent with the structures of deniagenin, denin and marsin as 3β, 11α, l2β, 14β, 17β, 20-hexahydroxy pregn-5-ene; desacylkondurangogenin C-3-*O*-α-D-glucopyranosyl-(1→4)-*O*-α-L-fucopyranoside and ketocalogenin-3-*O*-α-L-fucopyranoside, respectively.

Keywords: Asclepiadaceae; *Marsdenia roylei*; Desacylkondurangogenin C; Deniagenin; Denin; Marsin; Pregnane; Pregnane glycosides

1. Introduction

Pregnanes and their glycosides (Deepak et al., 1997) have been isolated from various plant species with most of them belonging to the Asclepiadaceae family. They have established themselves as an important class of biologically active compounds and have shown anti-tumor (Reichstein, 1967; Yoshimura et al., 1983; Hayashi et al., 1969, Duh et al., 1987), anti-cancer (Mitsuhashi et al., 1993; Ahsan et al., 1973), and anti-epilepsy (Mu et al., 1986) activities among others. In search for these novel compounds, various plants species of *Marsdenia* genus i.e. *M. condurango, M. formosa; M. incisia, M. koi, M. oreophila, M. tenacissima, M. thrysiflora* and *M. roylei* (family Asclepiadaceae) were examined. The different species *of Marsdenia* have shown varied biological activity. The crude extract of *M. condurango* cortex bark has been used as an avomatic bitter stomachic principle in popular medicine and also against cancer or syphilis (Hayashi et al., 1980). The condurango glycosides (CG) Ao, (CG) Bo, (CG) Co, (CG) Do, 20-iso-*O*-methyl condurango glycoside (CG) Co and 20-iso-*O*-methyl-condurango glycoside (CG) Do from *M. condurango* (Hayashi et al., 1981) were found active against Ehrlich ascites carcinoma, while two other condurango glycosides Eo1 and Eo2 have shown anti-carcinogenic activity (Mitsuhashi et al., 1993). Condurango glycosides A and C have cinnamoyl group in their aglycons, were the most potent differentiation inducers and M1 cells became phagocytic cells after 24 h treatment with these glycosides (Umehara et al., 1994). Six Condurango glycoside (Duh et al., 1987; Mitsuhashi et al., 1993; Ahsan et al., 1973; Mu et al., 1986) A, C, E2 and condurangoglycoside A, B, C obtained from the methanol extract of bark of *M. condurango* cortex possess differentiation inducing activity towards mouse myeloid leukemia (M1) cell line (Umehara et al., 1994). M1 cells were differentiated into phagocytic cells by these glycosides, which were found to be more effective than their aglycons. The pregnane glycosides obtained from *M. tenacissima* (Luo et al., 1993) showed cytotoxic, anti asthmatic, anti cancer, anti trachtices and fertility regulating activities. *M. koi* (Yuan et al., 1991, 1992) has shown good anti fertility activity. *M. roylei* (Kirtikar and Basu, 1984) is used as remedy in gonorrhoea.

In our previous studies on *M. roylei*, four oligosaccharides viz. rolinose, royleose, deniose and maryose (Kumar et al., 1999a,b) were isolated from chloroform soluble extract. The less polar fractions of the same extract that were not studied earlier were taken in our present studies resulting in the isolation of compound **1**, deniagenin, denin and marsin. This paper deals with the structure elucidation of these three novel pregnane and their glycosides.

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2. Results and discussion

2.1. Compound 1

This was analyzed for $C_{21}H_{36}O_5$ gave positive Libermann–Burchard test (Abisch and Reichstein, 1960) and also gave positive reaction with $NaIO_4$ (Sawleweiz et al., 1954), suggesting the presence of C-21 steroidal moiety with vicinal diol system in the molecule. Compound **1**, although isolated for the first time from natural resources, was confirmed to be 3β, 11α, 12β, 14β, 20-pentahydroxy pregnane with the help of FABMS, ^{1}H NMR of **1** and FABMS and ^{1}H, ^{13}C and ^{1}H–^{1}H COSY NMR experiments of its acetyl derivative (**2**). Compound **1** was found to be desacylkondurangogenin C that was prepared on alkaline hydrolysis of kondurangogenin C (Tschesche et al., 1967).

2.2. Deniagenin 3

Mp 110 °C. $[\alpha]_D$ −66.7°, $C_{21}H_{34}O_6$ gave positive Libermann–Burchardt test (Abisch and Reichstein, 1960) and tetra nitro methane test. The FAB mass spectrum of **3** showed the highest mass ion peak at m/z 405 which could be accounted for $(M+Na)^+$ showing that the molecular weight of **3** was 382, which is in accordance with the derived molecular formula of **3**, further suggesting that the **3** is a steroidal molecule having C-21 carbon skeleton with olefinic bond in it. The DBE value of five, derived from the molecular formula, indicated **3** to be a highly hydroxylated pregnane genin.

The ^{1}H NMR spectrum of **3** on addition of trichloroacetyl isocyanate reagent (Bose and Srinivasan, 1975) gave six signals which appeared at δ 8.33, 8.56, 8.89. 9.17, 9.61 and 10.33, respectively showing the presence of six hydroxyl groups, while its property to react with sodium periodate (Sawleweiz et al., 1954) indicated the presence of vicinal diol system in the molecule. Acetylation of compound **3** afforded an amorphous compound **4**. The ^{1}H NMR spectrum of deniagenin acetate **4** contained only four singlets of three protons each at δ 2.02, 2.05, 2.08 and 2.11 indicating the presence of four acylable hydroxyl groups in **3**, leading to the conclusion that the remaining two hydroxyl groups were tertiary in nature. As compound **3** did not isomerise with methanolic potassium hydroxide, it indicated the presence of a substituent, presumably a hydroxyl group, at C-17. A secondary methyl group doublet at δ 1.15 ($J=6$ Hz) along with one methine proton quartet at δ 4.88 ($J=6$ Hz) showed the presence of hydroxyl ethyl side chain at C-17. The presence of this quartet confirmed the presence of hydroxyl group at C-20.

The presence of a triplet at δ 5.59 ($J=10$ Hz) along with a doublet at δ 4.86 ($J=10$ Hz) in the ^{1}H NMR of **4** were assigned to the C-11 and C-12 methine proton, respectively, suggesting the presence of acetoxy groups at C-11 and C-12 of pregnane skeleton. The splitting pattern of C-11 and C-12 methine protons confirmed the diol system at C-11 and C-12 in it, which was indicated earlier by positive $NaIO_4$ test. The large value of their coupling constant 10 Hz confirmed their *trans* biaxial arrangement and fixed the configuration of the acetyl group at C-11 and C-12 as α and β, respectively (Singhal et al., 1980). The presence of acetoxy groups at C-11 and C-12 in **4** was further supported by the ^{1}H–^{1}H COSY NMR spectrum of **4**, which gave cross peaks at δ 5.59×4.85. A multiplet at δ 4.08 was assigned to the C-3 methine proton. The presence of four acylable hydroxyl groups at C-3, C-11, C-12 and C-20 was supported by ^{13}C NMR spectrum of **4** which gave signals at δ 71.24, 73.21, 73.85 and 78.04, respectively while the signals at δ 85.00 and 87.00 could be assigned for C-14 and C-17 bearing tertiary hydroxyl groups. A multiplet signal at δ 5.36 for H-6 showed the presence of a double bond at C-5 and C-6. The presence of double bond was further supported by its ^{13}C NMR spectrum showing signals at δ 119.00 and 142.00. The other important signals are given in the Table 1.

The ^{1}H NMR of **3** further supported the derived structure. It contained signals for C-11 and C-12 protons as triplet at δ 4.94 ($J=8$ Hz) and a doublet at δ 4.29 ($J=8$ Hz), respectively. The ^{1}H NMR also contained the signals for C-20 and C-3 protons, which appeared as a quartet at δ 4.15 ($J=7.5$ Hz) and a multiplet at δ 3.18, respectively. The C-6 vinylic proton appeared at δ 5.36 as a multiplet.

Table 1

Carbon no.	Desacylkondurangogenin C acetate (**2**) Chemical shift value (in δ)	Deninagenin acetate (**4**) Chemical shift value (in δ)
1	36.8	35.3
2	26.8	25.6
3	71.7	71.2
4	40.4	40.2
5	29.7	142.0
6	25.6	119.0
7	26.7	29.7
8	30.0	30.3
9	50.3	49.1
10	38.8	38.5
11	70.6	73.2
12	73.3	73.8
13	37.0	39.1
14	85.0	85.0
15	31.2	31.8
16	25.7	23.0
17	52.3	87.0
18	15.0	14.0
19	17.4	18.8
20	78.3	78.0
21	24.0	22.9

The FAB mass spectrum of **3** further substantiated the derived structure that contained the highest mass ion peak at *m/z* 405 which could be assigned for $(M+Na)^+$ thus supporting the molecular weight of **3** as 382. The mass spectrum also contained a peak at *m/z* 382 $(M)^+$. The loss of C-17 side chain as –$CHOHCH_3$, from molecular ion was confirmed by the mass ion peak at *m/z* 337 (M–$CHOHCH_3$). The characteristic retro-Diels Alder fission of C-5 double bond observed in pregn-5-enes gave peak at *m/z* 244 thus confirming the presence of hydroxyl groups at C-11, C-12, C-14, C-17 and C-20. The complementary ion peak at *m/z* 138 confirmed the presence of –OH group at C-3 of the pregnane. In the light of foregoing evidences the structure with configuration (Reichstein, 1967) of deniagenin **3** was established as 3β, 11α, 12β, 14β, 17β, 20-hexahydroxy pregn-5-ene.

2.3. Denin 5

$C_{33}H_{56}O_{14}$ mp 232 °C $[\alpha]_D+12.5°$ responded positively to Libermann–Burchardt (Abisch and Reichstein, 1960), test for steroids and Feigl (1975) and phenol sulphuric acid test (Dubois et al., 1956) for 6-deoxy and normal sugar. The FAB mass spectrum of **5** gave the highest mass ion peak at *m/z* 715 $(M+K)^+$ which was in accordance with the molecular formula $C_{33}H_{56}O_{14}$ ($M_r=676$). Two anomeric protons were observed as doublets at δ 4.98 and 5.46 in its ^{1}H NMR spectrum suggesting it to be a diglycoside. The monosaccharides present in glycoside **5** were identified by hydrolyzing it by the method of Killiani (1930) under forcing conditions which afforded a mixture of two sugars **9** and **10**, identified as D-glucose and L-fucose respectively by comparison with authentic samples ($[\alpha]_D$, PC and TLC). The aglycone could not be isolated as it was destroyed under the strong acidic conditions employed in the Killiani (1930) hydrolysis.

To identify the native genin and also to determine the sequence of sugars in it, **5** was subjected to mild acid hydrolysis at room temperature, using the method of Mannich and Siewert (1942) and monitoring the reaction on TLC and PC. After 5 days of hydrolysis, the reaction mixture contained unreacted **5**, and a new spot, which was presumably the monoglycoside **8** besides glucose (PC, TLC), which indicated that glucose was the terminal sugar unit in **5**. The hydrolysis was complete in 10 days affording chromatographically pure products **7**, **9** and **10**. Compounds **9** and **10** were identified as glucose and fucose by comparison with authentic samples ($[\alpha]_D$, PC and TLC) respectively. The compound **7** was identified as desacylkondurangogenin C (**1**), It was, therefore, concluded that in glycoside **5**, the disaccharide glycon was glycosidically linked to **1** through L-fucose, which was further linked with D-glucose at non-reducing end. For convenience, the two monosaccharide units of **5** were designated as S_2 and S_1, respectively starting from non reducing end.

The ^{1}H NMR spectrum of **5** contained two doublets of one proton each at δ 4.98 ($J=3$ Hz) and 5.46 ($J=3$ Hz), which were assigned to anomeric protons of α-glucose and α-fucose, (S_2 and S_1), respectively. The small coupling constants of anomeric doublets showed that the D-glucose and L-fucose were linked through α-glycosidic linkage in their $^4C_1$ and $^1C_4$ conformations (Dubois et al., 1956), respectively. The ^{1}H NMR spectrum of **5** also contained the two secondary methyl groups doublets at δ 1.14 ($J=6.3$ Hz) and 1.27 ($J=6.9$ Hz) for C-6 methyl group of L-fucose and C-21 methyl group of **1**, respectively. The methine protons of C-11, C-12 and C-20 of aglycon appeared as triplet at δ 4.08 ($J=7$ Hz), doublet at 4.12 ($J=7$ Hz) and multiplet at 3.82, respectively. The chemical shifts of these protons were in resemblance with the chemical shifts of C-11, C-12 and C-20 methine protons of **1** while the C-3 methine proton of **5** appeared as multiplet at δ 4.28 which was shifted downfield in comparison to the C-3 proton of **1** showing that sugar S_1–S_2 (**6**) was linked to aglycon at C-3 and the shift was due to the glycosylation at C-3. The glycosidation of C-3 hydroxyl was further confirmed by the ^{1}H NMR spectrum of nona-*O*-acetyl denin, **11** which contained the nine acetyl group signals at δ 1.91, 1.93, 1.97, 2.01, 2.02, 2.04, 2.06, 2.08 and 2.13 besides the downfield shifted methine protons of C-11 as triplet at δ 5.09 ($J=9$ Hz), C-12 as doublet at δ 4.75 ($J=8$ Hz) and C-20 as multiplet at δ 4.95 respectively. However after acetylation, the C-3 proton of **11** did not shift, confirming that the disaccharide (S_1+S_2) **6** was linked to aglycon at C-3 of **1**. Further, the interglycosidic linkage between (S_1 and S_2) was also confirmed by the chemical shift of H-4 of S_1. As the H-4 of S_1 appeared at the same chemical shift in the ^{1}H NMR spectrum of acetylated denin **11** and denin **5** i.e. δ 4.04 and 4.05 respectively, it clearly shows that the H-4 of S_1 was not acetylated due to its involvement in the glycosidation confirming the 1→4 glycosidic linkage between S_1 and S_2.

The FAB mass spectrum of **5** showed the highest mass ion peak at *m/z* 715 $(M+K)^+$, 699 $(M+Na)^+$ and at *m/z* 677 $(M+H)^+$. The molecular ion after the loss of C-17 side chain (CH_3CHOH) gave fragment ion at *m/z* 631. The FAB mass spectrum of **5** also showed the fragment ion for sugar component i.e. $[disaccharide]^+$ at *m/z* 326 and aglycon fragment, i.e. at *m/z* 369 for $(aglycon+H)^+$. The sequence of the monosaccharides in **5** was supported by the mass ion peak at *m/z* 497 which was due to monoglycoside comprising of genin + fucose showing that the fucose was directly linked to aglycon and glucose was present at the terminal end. The FAB mass spectrum of **5** also contained the other mass fragments characteristic of sugar and aglycon. In the light of foregoing evidences, the structure of **5** was

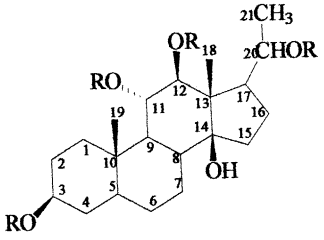
defined as desacylkondurangogenin C-3-*O*-α-D-glucopyranosy1-(1→4)-*O*-α-L -fucopyranoside.

2.4. Marsin **12**

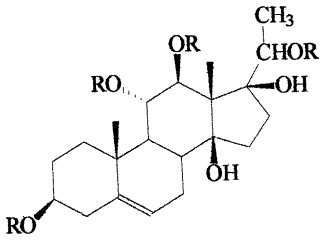
Mp 142 °C, $C_{27}H_{42}O_7$ $[\alpha]_D$ +6.7° responded positively to the Libermann–Burchardt test (Abisch and Reichstein, 1960) for steroids and Feigl (1975) test for 6-deoxy sugar suggesting it to be a steroidal glycoside of 6-deoxy sugar. The FAB mass spectrum of **12** gave the highest mass ion peak at *m/z* 540 which could be assigned for quasimolecular ion, i.e. $[M+Na+K]^+$ showing the molecular weight of compound to be 478 which was in accordance with the derived molecular formula $C_{27}H_{42}O_7$. The ^1H NMR spectrum of **12** contained one anomeric proton signal, which appeared as a doublet at δ 5.44 suggesting it to be a monoglycoside.

To identify the genin and sugar in **12**, it was subjected to mild acid hydrolysis with TLC and PC monitoring, using the method of Mannich and Siewert (1942). The hydrolysis was complete in six days showing two spots, which on column chromatography afforded two pure products **13** and **10**. Compound **10** was identified as L-fucose on comparison with authentic sample ($[\alpha]_D$, PC and TLC). Compound **13**, $C_{23}H_{32}O_3$ gave positive Libermann–Burchardt test (Abisch and Reichstein, 1960) and underwent $NaBH_4$, reduction (Bhatnagar et al., 1968) showing it to be a steroidal moiety having keto methyl side chain in it, which was identified as ketocalogenin (Deepak et al., 1997) on comparison with authentic sample (mmp, $[\alpha]_D$, and TLC).

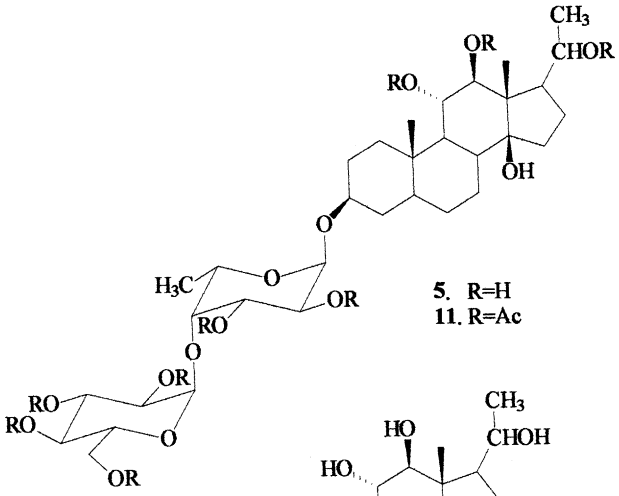
The ^1H NMR spectrum of **12** contained a doublet of one proton at δ 5.44 ($J=3$ Hz) which was assigned to the anomeric proton of fucose. The small coupling constant of anomeric proton indicated the presence of α-glycosidic linkage in $^1C_4$ conformation (Dubois et al., 1956). A doublet of three protons at δ 1.26 ($J=7$ Hz) was due to secondary methyl group of fucose. The fucose was linked to the aglycon at C-3, which was the only available position for glycosidation. In aglycon **13** a singlet of three protons at δ 2.25 was reported due to the methyl group signal of ketomethyl side chain pre-



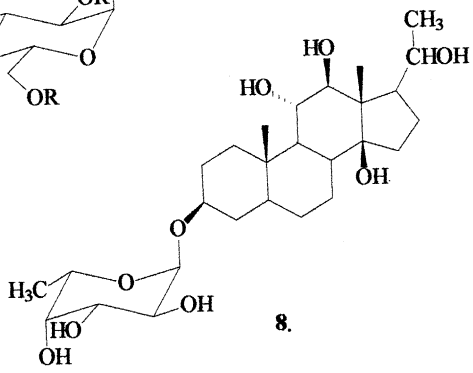
1. R=H
2. R=Ac



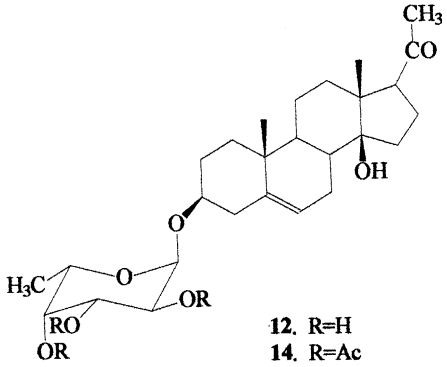
3. R=H
4. R=Ac



5. R=H
11. R=Ac



8.



12. R=H
14. R=Ac

sent at C-17 position of the aglycon. The glycosidation of C-3 hydroxyl group was further confirmed by the ^{1}H NMR spectrum of tri-*O*-acetyl marsin **14** which contained the three acetyl group signals at δ 1.95, 1.99, and 2.03. The C-3 methine proton of **12** did not show any change in its chemical shift confirming that the fucose was linked to aglycon at C-3 of ketocalogenin. The methyl group signal of three proton of the ketomethyl side chain of the aglycon remained at δ 2.25.

The FAB mass spectrum of **12** showed the highest mass ion peak at *m*/*z* 540 which could be assigned for $[M+Na+K]^+$. The molecular ion after the loss of C-17 side chain (CH_3CO) gave fragment ion peak at *m*/*z* 435. The FAB mass spectrum of **12** also showed the fragment ion for sugar component, i.e. $[fucose+H]^+$ at *m*/*z* 165 and aglycon fragment i.e. at *m*/*z* 355 for $[M+Na]^+$. The FAB mass spectrum also contained the other mass ion fragments characteristic of sugar and aglycon. In the light of foregoing evidences, the structure of marsin **12** was deduced as ketocalogenin-3-*O*-α-L-fucopyranoside.

3. Experimental

General procedures were same as reported earlier (Kumar et al., 1999a,b). ^{1}H and ^{13}C NMR spectra were recorded on a 300 MHz (Bruker) spectrometer in $CDCl_3$ with TMS as internal standard. FAB mass spectrum were recorded with JEOL mass spectrometer model JMS-SX 102 FAB with DA 6000 data system and Jeol mass spectrometer D-300 with IMA-2000 data system respectively. TLC was performed on silica gel G (Qualigens) and column chromatography was done over silica gel 60–120 mesh (Qualigens). Normal sugars were made visible by Partridge reagent on PC.

3.1. Plant extraction

The aerial part of plant *M. roylei* (40 kg) was collected from Dehradun, U.P., India. The identity of the plant was confirmed by Dr. S. C. Saini, Botanist, Birbal Sahani Institute of Paleobotany, Lucknow, India, where a voucher specimen (No. BSIP-11870) was deposited. Shade dried powdered plant material (8 kg) was extracted by the method used for pregnane glycosides (Khare et al., 1984) using 50–95% aq. EtOH. The ethanolic extracts were concentrated under red. pres. and the concentrate was exhaustively extracted successively with hexane, $CHCl_3$, $CHCl_3$–EtOH (4:1) and $CHCl_3$–EtOH (3:2). These extracts on evaporation yielded the following quantities of residues: hexane (0.67 g), $CHCl_3$ (7.90 g), $CHCl_3$–EtOH (4:1) (3.50 g) and $CHCl_3$–EtOH (3:2) (4.34 g). Repeated column chromatography of $CHCl_3$ soluble extract using $CHCl_3$–MeOH of varied concentration as eluant over silica gel column yielded compound **1**, deniagenin (**3**), denin (**5**) and marsin (**12**).

3.2. Compound *1*

Mp 127 °C (white amorphous powder), $[\alpha]_D$, +135.6° (*c*, 0.14; $CHCl_3$), (found: C, 68.39; H, 9.84% $C_{21}H_{36}O_5$ requires C, 68.4%; H, 9.85%). It gave a positive Libermann–Burchardt test and underwent $NaIO_4$ oxidation. ^{1}H NMR δ 1.03 (3H, *s*, 19 Me), 1.09 (3H, *s*, 18 Me), 1.16 (3H, *d*, *J* = 6 Hz; 21 Me), 3.07 (1H, *m*, H-3), 3.82 (1H, *m*, H-20), 4.08 (1H, *t*, *J* = 7 Hz, H-11), 4.12 (1H, *d*, *J* = 7 Hz, H-12). FABMS: *m*/*z* 391 $(M+Na)^+$, 369 $(M+H)^+$, 351 $(369\text{-}H_2O)^+$, 324 $(369\text{-}CHOH\ CH_3)^+$, 307 $(324\text{-}OH)^+$, 289 $(307\text{-}H_2O)^+$, 271 $(289\text{-}H_2O)^+$, 254 $(271\text{-}OH)^+$, 228 $(368\text{-}C_9H_{16}O)^+$, 210 $(228\text{-}H_2O)^+$, 165 $(210\text{-}CH_3CHOH)^+$, 150 $(165\text{-}CH_3)^+$, 140 $(M+\text{-}228)^+$, 133 $(150\text{-}OH)^+$, 122 $(140\text{-}H_2O)^+$, 107 $(122\text{-}CH_3)^+$, 97 $(142\text{-}CH_3CHOH)^+$.

3.3. Deniagenin *3*

Mp 110 °C (white amorphous powder), $[\alpha]_D$ −66.7° (*c*, 0.3, $CHCl_3$), (found: C, 68.41; H, 9.83% $C_{21}H_{34}O_6$ requires C, 68.45%; H, 9.85%). It gave a positive Libermann–Burchardt and tetra nitro methane tests and underwent $NaIO_4$ oxidation. ^{1}H NMR δ 0.89 (3H, *s*, 19 Me), 1.15 (3H, *d*, *J* = 6 Hz; 21-Me), 1.22 (3H, *s*, 18 Me), 3.18 (1H, *m*, H-3), 4.29 (1H, *d*, *J* = 8 Hz, H-12), 4.88 (1H, *q*, *J* = 7.5 Hz, H-20), 4.94 (1H, *t*, *J* = 8 Hz, H-11), ad 5.36 (1H, *m*, H-6). ^{13}C NMR data are given in Table 1. FABMS: *m*/*z* 405 $(M+Na)^+$, 337 $(M^+\text{–}CH_3CHOH)^+$, 307 $(337\text{-}2CH_3)^+$, 291 $(363\text{-}CH_3CHOHCH=CH_2)^+$, 289 $(307\text{-}H_2O)^+$, 273 $(29l\text{-}H_2O)^+$, 271 $(289\text{-}H_2O)^+$, 253 $(27l\text{-}H_2O)^+$, 244 $(382\text{-}C_9H_{14}O)^+$, 226 $(244\text{-}H_2O)^+$, 225 $(253\text{-}C_2H_4)^+$, 208 $(226\text{-}H_2O)^+$, 207 $(225\text{-}H_2O)^+$, 199 $(244\text{-}CH_3CHOH)^+$, 191 $(208\text{-}OH)^+$, 182 $(199\text{-}OH)^+$, 154 $(182\text{-}C_2H_4)^+$, 138 $(M+\text{-}244)^+$, 136 $(154\text{-}H_2O)^+$, 120 $(138\text{-}H_2O)^+$, 105 $(120\text{-}CH_3)^+$, 119 $(191\text{-}CH_3CHOH\text{-}CH=CH_2)^+$.

3.4. Denin *5*

Mp 232 °C (white amorphous powder), $[\alpha]_D$ +12.5° (*c*, 0.016; MeOH), (found: C, 58.52; H, 8.30% $C_{33}H_{56}O_{14}$ requires C, 58.56%; H, 8.33%). It gave positive tests in phenol sulphuric acid, Feigl and Libermann–Burchardt reactions. It underwent $NaIO_4$ oxidation. ^{1}H NMR δ 0.97 (3H, *s*, 18 Me), 1.07 (3H, *s*, 19 Me), 1.14 (3H, *d*, *J* = 6.3 Hz; 6′ Me-fucose), 1.27 (3H, *d*, *J* = 6.9 Hz; 21-Me), 3.82 (1H, *m*, H-20), 4.05 (1H, *m*, H-4'S_1), 4.08 (1H, *t*, *J* = 7 Hz, H-11), 4.12 (1H, *d*, *J* = 7 Hz, H-12), 4.28 (1H, *m*, H-3), 4.98 (1H, *d*, *J* = 3 Hz, H-1″ glu), 5.46 (1H, *d*, *J* = 3 Hz, H-1′, fuc). FABMS: *m*/*z* 715 $[M+K]^+$, 699 $[M+Na]^+$, 677 $(M+H)^+$, 662 $(677\text{-}CH_3)^+$, 647 $(677\text{-}2CH_3)^+$, 631 $(M^+\text{–}CH_3CHOH)^+$ 616 $(631\text{-}CH_3)^+$, 604

(676-$CH_3CHOHCH=CH_2$)$^+$, 602 (647-CH_3CHOH)$^+$, 601 (616-CH_3)$^+$, 590 (662-CH_3CHOH–$CH=CH_2$)$^+$, 584 (602-H_2O)$^+$, 566 (584-H_2O)$^+$, 497 (677-glucose)$^+$, 479 (497-H_2O)$^+$, 461 (479-H_2O)$^+$, 401 (461-CH_2OH–CHO)$^+$, 383 (401-H_2O)$^+$, 369 (aglycon + H)$^+$, 368 (383-CH_3)$^+$, 326 (disaccharide ion)$^+$, 324 (genin–CH_3CHOH)$^+$, 308 (disaccaride–H_2O)$^+$, 307 (324-OH)$^+$, 295 (369-$CH_3CHOHCH=CH_2$)$^+$, 289 (307-H_2O)$^+$, 290 (326-2H_2O)$^+$, 273 (290-OH)$^+$, 271 (289-H_2O)$^+$, 264 (308-CH_3CHO)$^+$, 254 (271-OH)$^+$, 248 (308-CH_2OH–CHO)$^+$, 230(248-H_2O)$^+$, 228 (368-$C_9H_{16}O$)$^+$, 225 (369-$C_8H_{14}O_2$)$^+$, 213 (273-CH_2OH–CHO)$^+$, 207 (225-H_2O)$^+$, 210 (228-H_2O)$^+$, 186 (230-CH_3CHO)$^+$, 165 (210-CH_3CHOH)$^+$, 150 (165-CH_3)$^+$, 133 (150-OH)$^+$, 122 ($C_9H_{16}O$-H_2O)$^+$, 116 (133-OH)$^+$, 107 (122-CH_3)$^+$, 97 (142-CH_3CHOH)$^+$.

3.5. Marsin **12**

Mp 142 °C (white amorphous powder), $[\alpha]_D$ +6.70 (*c*, 0.03; $CHCl_3$), (found C, 67.71; H, 8.42% $C_{27}H_{47}O_7$ requires C, 67.75; H, 8.44%). It gave positive Feigl, Libermann–Burchardt reactions and tetra nitro methane test. ^{1}H NMR δ 1.01 (3H, *s*, 19 Me), 1.02 (3H, *s*, 18 Me), 1.26 (3H, *d*, $J=7$ Hz; 6′ Me-Fuc), 2.25 (3H, *s*, H-21), 3.66 (1H, *m*, H-3), 5.22 (1H, *m*, H-6), 5.44 (1H, *d*, $J=3$Hz, H-1′ fuc). FABMS: *m*/*z* 540 (M + Na + K)$^+$, 478 (M)$^+$, 460 (478-H_2O)$^+$, 442 (460-H_2O)$^+$, 399 (442-CH_3CO)$^+$, 408 (478-CH_3CO-$CH=CH_2$)$^+$, 391 (408-OH)$^+$, 373 (391-H_2O)$^+$, 329 (373-CH_2CHO)$^+$, 435 (M–CH_3CO)$^+$, 408 (425-OH)$^+$, 391 (408-OH)$^+$, 463 (478-CH_3)$^+$, 355 (genin + Na)$^+$, 337 (355-H_2O)$^+$, 307 (337-2CH_3)$^+$, 289 (307-H_2O)$^+$, 274 (289-CH_3)$^+$, 262 (332- $CH_3COCH=CH_2$)$^+$, 232 (262-2CH_3)$^+$, 214 (232-H_2O)$^+$, 194 (332-$C_9H_{14}O$)$^+$, 192 (332-$C_8H_{12}O_2$)$^+$, 176 (194-H_2O)$^+$, 151 (194-CH_3CO)$^+$, 138 (332-$C_{12}H_{18}O_2$)$^+$, 134 (151-OH)$^+$, 133 (176-CH_3CO)$^+$, 120(138-H_2O)$^+$, 105 (120-CH_3)$^+$, 97 ($C_8H_{12}O_2$–CH_3CO)$^+$.

3.6. Mannich hydrolysis of **5**

To a solution of **5** (10 mg) in Me_2CO (2 m1), conc. HCl (0.02 m1) was added. After 5 days, the reaction mixture showed a new spot identical to glucose. Hydrolysis was complete after 10 days showing three spots identified as **1**, L-fucose, and D-glucose (TLC, PC, $[\alpha]_D$). Usual work up followed by column chromatography gave **1** (2.1 mg), $[\alpha]_D$, +135.6° (*c*, 0.11, $CHCl_3$), D-glucose (1.5 mg), $[\alpha]_D$ +52.2° (*c*, 0.10, H_2O), and L-fucose (1.3 mg), $[\alpha]_D$ +74.4° (*c*, 0.11, H_2O).

3.7. Killiani hydrolysis of **5**

The compound **5** (10 mg) was treated in 1 ml killiani mixture (3.5 parts AcOH + 5.5 parts H_2O + 1 part conc. HCl) in a hydrolysis flask. It was heated at 100 °C for 1 h. TLC monitoring showed two spots identified as D-glucose and L-fucose. Usual working followed by column chromatography gave D-glucose (2.1 mg), $[\alpha]_D$ +52.31° (*c*, 0.13, H_2O), and L-fucose (1.7 mg), $[\alpha]_D$ +74.4° (*c*, 0.10, H_2O).

3.8. Mannich hydrolysis of **12**

To a solution of **12** (10 mg) in Me_2CO (3 ml), conc. HC1 (0.02 ml) was added. The hydrolysis was monitored on TLC and PC. The hydrolysis was complete in 6 days showing two new spots. The polar spot was identical to L-fucose by comparison with authentic sample. The less polar spot was identified as ketocalogenin. Usual work up followed by column chromatography gave ketocalogenin (3.2 mg), mp 220–222°, $[\alpha]_D$ +47.2° (*c*, 0.14; $CHCl_3$), and L-fucose (2.8 mg), $[\alpha]_D$ +74.3° (*c*, 0.12, H_2O).

3.9. Tetra O-acetyl derivative of **1**, (**2**)

Compound **1** (3.5 mg) was dissolved in dry pyridine (0.3 ml) and mixed with Ac_2O (0.3 ml). The mixture was heated on a water bath at 100 °C for 1 h. Usual work up after the removal of excess of Ac_2O gave (**2**), $C_{29}H_{44}O_9$, $[\alpha]_D$ +13.3° (*c*, 0.14, $CHCl_3$). ^{1}H NMR δ 1.16 (*d*, $J=6$ Hz, H-21), 2.00 (3H, *s*, OAc), 2.03 (3H, *s*, OAc), 2.05 (3H, *s*, OAc), 2.08 (3H, *s*, OAc), 4.08 (1H, *m*, H-3), 4.83 (1H, *d*, $J=8$ Hz, H-12), 4.92 (1H, *m* H-20) and 5.09 (1H, *t*, $J=8$ Hz, H-11). These assignments were also confirmed by its ^{1}H–^{1}H COSY NMR spectrum.

3.10. Tetra-O-acetyl deniagenin (**4**)

Compound **3** (4 mg) was dissolved in dry pyridine (0.4 ml) and mixed with Ac_2O (0.4 ml). The mixture was heated on a water bath at 100 °C for 1 h. Usual work up after the removal of excess of Ac_2O gave **4**, $C_{29}H_{42}O_{10}$, $[\alpha]_D$ +18.33° (*c*, 0.11, $CHCl_3$). ^{1}H NMR δ 1.15 (3H, *d*, $J=6$ Hz, 21 CH_3), 2.02, 2.05, 2.08, 2.11 (12H, 4s, 4xOAc), 4.08 (1H, *m*, H-3), 4.86 (1H, *d*, $J=10$ Hz, H-12), 4.88 (1H, *q*, $J=6$ Hz, H-20), 5.36 (1H, *m*, H-6) and 5.59 (1H, *t*, $J=10$ Hz, H-11).

3.11. Nona-O-acetyl denin (**11**)

Compound **5** (5 mg) was dissolved in dry pyridine (0.5 ml) and mixed with Ac_2O (0.5 ml). The mixture was heated on a water bath at 100 °C for 1 h. Usual work up after the removal of excess of Ac_2O gave **11**, $C_{51}H_{74}O_{23}$, $[\alpha]_D$ +15.50 (*c*, 0.14, $CHCl_3$). ^{1}H NMR δ 1.91, 1.93, 1.97, 2.01 2.02, 2.04, 2.06, 2.08, 2.13 (27H, 9s, 9xOAc), 4.04 (1H, *m*, H-4′, S1), 4.26 (1H, *m*, H-3), 4.95 (1H, *m*, H-20), 4.75 (1H, *d*, J = 9 Hz, H-12), 4.83 (1H, $J=3$ Hz, H-1″, α-Glu), 5.09 (1H, *t*, $J=9$ Hz, H-11) and 5.50 (1H, d, $J=3$ Hz, H-1′ α-fuc).

3.12. Tri-O-acetyl marsin (**14**)

Compound **12** (4 mg) was dissolved in dry pyridine (0.4 ml) and mixed with Ac_2O (0.4 ml) The mixture was heated on a water bath at 100 °C for 1 h. Usual work up after the removal of excess Ac_2O gave **14**, $C_{33}H_{48}O_{10}$, $[\alpha]_D$ +163.6° (*c*, 0.15, $CHCl_3$). ^1H NMR δ 1.05 (3H, *s*, H-19), 1.13 (3H, *s*, H-18), 1.26 (1H, *d*, $J=1$ Hz, H-6′), 1.95, 1.99, 2.03 (9H, 3s, 3xOAc), 2.25 (3H, *s*, H-21).

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References

Abisch, E., Reichstein, T., 1960. Orientierende Chemisehe Untersuchung Einiger Apocynaceae. Helv. Chim. Acta 43, 1844–1861.

Ahsan, A.M., Piatak, D.M., Sorenson, P.D., 1973. Isolation and structure of amplexoside A. A new glycoside from *Asclepias amplexicaulis*. Experientia 29, 788–789.

Bhatnagar, A.S., Stocklin, W., Reichestein, T., 1968. Die Pregnanderivate des samen von *Dregea abyssinica* (HOCHST) K.SCHUM (Asclepiadaceae) II Structturbestimmungen Glykoside und Aglykone, 305, mitteilung. Helv. Chim. Acta 5, 133–147.

Bose, A.K., Srinivasan, P.R., 1975. Trichlorcacetylisocynates as an in situ derivatizing reagent for ^{13}C NMR. Tetrahedron 31, 3025.

Deepak, D., Srivastav, S., Khare, A., 1997. Pregnane glycosides. Prog. Chem. Nat. Prod. 71, 169–325.

Dubois, M., Golles, K.A., Hamilton, J.K., Rthers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–357.

Duh, C.Y., Pezzuto, J.M., Kinghorn, A.D., Leuang, S.L., Farnsworth, N.R., 1987. Plant anticancer agents. XLIV: cytotoxic constituents from *Stizophyllium riparium*. J. Nat. Prod. 50, 63–74.

Feigl, F., 1975. Spot Tests in Organic Analysis, seventh ed. Elsevier Publications, Amsterdam.

Hayashi, K., Nakao, A., Mitsuhashi, H., 1969. Studies on the constituents of Asciepiadaceac plants (XXVI) Isolation of a new glycoside from *Dregea volubilis*. Chem. Pharm. Bull. 17, 2629–2632.

Hayashi, K., Wada, K., Mitsuhasi, H., Bando, H., Takase, M., Terada, S., Koide, Y., Aiba, T., Narita, T., Mizuno, D., 1980. Antitumor active glycosides from *Condurango cortex*. Chem. Pharm. Bull. 28, 1954–1958.

Hayashi, K., Wada, K., Mitsuhasi, H., Bando, H., Takase, M., Terada, S., Koide, Y., Aiba, T., Narita, T., Mizuno, D., 1981. Further investigation of anti-tumor condurango glycosides with C-18 oxygenated aglycone. Chem. Pharm. Bull. 29, 2725–2730.

Khare, N.K., Khare, M.P., Khare, A., 1984. Two pregnane ester glycosides from *Pergularia pallida*. Phytochemistry 23, 2931–2935.

Killiani, H., 1930. Uber Digitalinum Verum. Bet. Deutsch Chem. Ges 63, 2866–2869.

Kirtikar, K. R., Basu, B. D., 1984. Indian Medicinal Plants, Vol III, p.1628.

Kumar, A., Khare, N.K., Khare, A., 1999. Two oligosaccharides from *Marsdenia roylei*. Phytochemistry 50, 1353–1357.

Kumar, A., Khare, N.K., Khare, A., 1999. Trisaccharides from *Marsdenia roylei*. Phytochemistry 52, 675–679.

Luo, S.Q., Lin, Z.L., Cordell, G.A., Xue, L., Johnson, M.E., 1993. Polyoxypregnanes from *Marsdenia tenacissima*. Phytochcmistry 34, 1615–1620.

Mannich, C., Siewert, O., 1942. Uber g-Strophanthin (Ouabain) and g-Strophanthidine. Chem. Ber. 75, 737–755.

Mitsuhashi, H., Mizuno, D., Hayashi, K., Abe, S., Takase, M., Narita, T., 1993. Chem. Abstr. 98, 132306.

Mu, Q., Lu, J., Zhou, Q., 1986. Two new antiepilepsy compounds-Ottophyllosides A and B. Sci Sin. Ser. B 29, 295 (Chem. Abstr. 1987, 106, 81545).

Reichstein, T., 1967. Cardenolid- und Pregnaneglykoside. Naturwissenschaften 54, 53–66.

Sawleweiz, E., Weiss, Ek., Reichstcin, T., 1954. Die Glykoside von Peiploca nigrescens Afzel'Glykoside und aglykone 134. Mitterlung. Helv. Chim. Acta 37, 1004–1036.

Singhal, S., Khare, M.P., Khare, A., 1980. Cissogenin, a pregnane genin from *Marsdenia tenacissima*. Phytochemistry 19, 2427–2430.

Tschesche, R., Kohl, H., Welzl, P.K., 1967. Digitanol glycosides. XVI. Structure of kondurangenins A and C_2. Tetrahedron 23, 1461–1471.

Umehara, K., Endoh, M., Miyase, T., Kuroyangi, M., Ueno, A., 1994. Studies on differentiation inducers. IV. Pregnane derivatives from *Condurango cortex*. Chem. Pharm. Bull. 42, 611–616.

Yoshimura, S.I., Narita, H., Hayashi, K., 1983. Mitsuhashi, H, Studies on the constituents of Asclepiadaceae plants LVI. Isolation of new anti tumour active glycoside from *Dregea volubilis* (L). Benth. Chem. Pharm. Bull 31, 3971–3983.

Yuan, J.L., Ding, W.P., Shi, J.P., Lu, Z.Z., Zhou, B.N., Erdelmeier, C.A., Cordell, G.A., Fong, H.H.S., Farnsworth, N.R., 1991. J. Tomng Ji. Med. Univ 11, 165.

Yuan, J.L., Lu, Z.Z., Che, G.X., Ding, W.P., Zhou, B.N., Erdelmeier, C.A.J., Hamburger, M.O., Fong, H.H.S., Cordell, G.A., 1992. The pregnane glycoside marsdekoiside A from *Marsdenia koi*. Phytochemistry 31, 1058–1060.