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Three Novel Pregnane Glycosides from *Leptadenia reticulata* Wight and Arn.

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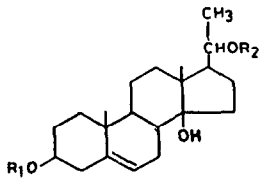
Key Words: Leptadenia reticulata; Asclepiadaceae; Pregnane glycoside: Reticulin; Deniculatin; Leptaculatin

Abstract: The structure of three novel pregnane glycosides viz. Reticulin (1), Deniculatin (2) and Leptaculatin (3) isolated from *Leptadenia reticulata* (fam. Asclepiadaceae) were elucidated with modern physico-chemical methods and chemical transformations. (1), (2) and (3) were defined as calogenin-3-O- β-cymaropyranosyl-(1→4)-O-3-O-methyl-α-D- galactopyranosyl-(1→4)-O-β-D-digitoxopyranosyl- (1→4)-O-β-D-cymaropyranoside, calogenin-3-O-3-O- methyl-α-D-galactopyranosyl-(1→4)-O-β-D- digitoxopyranoside and calogenin-3-O-β-D-glucopyranosyl- (1→4)-O-β-D-glucopyranosyl-(1→4)-O-β-D- cymaropyranoside, respectively.

Plants belonging to family Asclepiadaceae are rich source of biologically active cardiac[1] and pregnane glycosides[2] which are known to possess anti-tumour and anticancer[3] activity. Leaves of *L. reticulata* (Wight and Arn) are used in asthma and cough[4] in folk medicine and has also shown to possess lactogenic property.[4] Previous chemical studies of the plant showed the presence of flavonoids, triterpenes and steroids.[5,6] This paper reports the isolation and structure elucidation of three novel pregnane glycosides (**1**), (**2**) and (**3**) from the combined chloroform- chloroform:ethanol (4:1) extracts by repeated column chromatography.

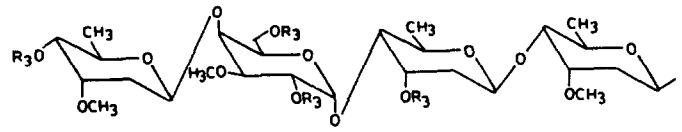
RESULTS AND DISCUSSION

Reticulin (**1**), $C_{48}H_{80}O_{17}$ (positive-ion FABMS: m/z 929 [M^++1]), mp 119-122°C $[\alpha]_D$-7.1°, responded positively to Liebermann- Burchardt[7] test for steroids, and xanthydrol[7] and Keller-Kiliani[7] reactions for 2,6-dideoxy sugar indicating it to be a steroidal glycoside with 2-deoxy sugar(s). It showed four anomeric carbons



	R_1	R_2	R_3
(1)	R_a	H	H
(2)	R_b	H	H
(3)	R_c	H	H
(4)	H	H	-
(6)	R_d	H	-
(7)	R_e	H	-
(8)	R_a	Ac	Ac
(9)	R_b	Ac	Ac
(11)	R_c	Ac	Ac

R_a =



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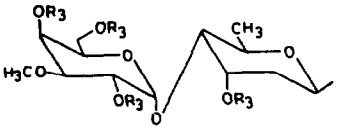

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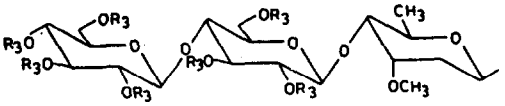

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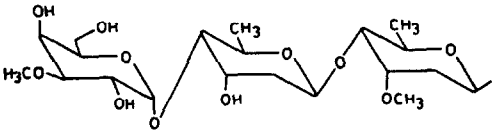

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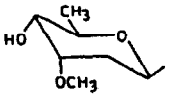
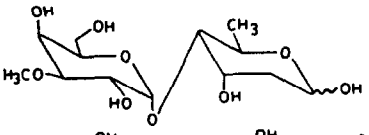
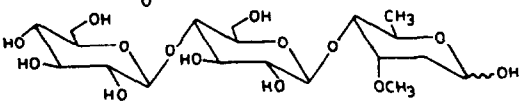


(5)



(10)



at δ 103.7, 103.0, 102.4 and 99.3 in its ^{13}C NMR spectrum and four anomeric protons at δ 5.16 (1H), 4.77 (1H) and 4.73 (2H) in its ^{1}H NMR spectrum at 400 MHz suggesting it to be a tetraglycoside. Besides, the ^{1}HNMR spectrum also showed three singlets of three protons each at δ 3.52, 3.49 and 3.46 for three methoxy functions and methylene group signals in the region δ 2.30-2.36 (3H) and 1.81-1.88 (3H) for the respective equatorial and axial protons of 2-deoxy sugars, indicating the presence of three 2-deoxy sugars in tetraglycoside (**1**).

The aglycon and monosaccharides of (**1**) were identified by hydrolysing it under mild acid (conc. HCl:Acetone, 1:100) condition using the method of Mannich and Siewert.[8] After 3 days one new spot appeared which was identical to D-cymarose (TLC, PC). After 7 days two new spots appeared which were identified as calogenin (**4**)[7] and presumably the disaccharide (**5**) (TLC) with the complete consumption of original glycoside (**1**). Hydrolysis was complete after 10 days showing four spots on TLC which were identified as calogenin (**4**), D-cymarose,[9] D- digitoxose[7] and 3-O-methyl-D-galactose[10] with the authentic samples (TLC, PC, $[\alpha]_D$). D-cymarose and D-digitoxose were further characterized by preparing their respective acid phenyl hydrazides[9,7] while 3-O-methyl-galactose was converted to methyl-3-O-methyl-α-D-galactopyranoside[10] and compared with its synthetic sample (TLC, PC, $[\alpha]_D$). The sequence of the sugars in (**1**) was established by hydrolysing it under very mild acid conditions (0.005 N H_2SO_4 at room temperature). After 10 days, reaction mixture exhibited three spots which were identified as D-cymarose (TLC), presumably a triglycoside (**6**) and starting material (**1**) showing that D-cymarose was at the terminal end. After 15 days, two new spots (TLC) appeared which were probably of disaccharide (**5**) and monoglycoside (**7**). The hydrolysis was complete after 20 days, showing four spots identified as calogenin (**4**), D-cymarose, D-digitoxose and 3-O-methyl-D-galactose (TLC, PC). Thus, the disaccharide (**5**), which is made up of 3-O- methyl-D-galactose and D-digitoxose, the latter being at reducing end, is second in sequence after the terminal D-cymarose. It further suggested that the sugar chain is glycosidically linked to the aglycon (**4**) through remaining methoxy-2,6-dideoxy sugar i.e. D-cymarose. For convenience, the four monosaccharides of (**1**) were designated as S_4, S_3, S_2 and S_1, respectively, starting from the terminal end.

The ^{1}H NMR spectrum of (**1**) showed a one proton doublet at δ5.16 (J=4 Hz) and two double-doublets at δ4.77 (1H, J=8 and 2 Hz) and 4.73 (2H, J=9 and 2 Hz) due to anomeric protons of S_3, the two units of D-cymarose (S_4 and S_1) and S_2 respectively. The large coupling constants of the two double- doublets showed the presence of 2-deoxy sugars S_4, S_2 and S_1 in $^{4}C_1$ (D)[11] conformation linked through β-glycosidic linkages. The small coupling constant of the doublet due to S_3 showed an α-glycosidic linkage present in $^{4}C_1$ (D) confirmation. The ^{13}C NMR spectrum of (**1**) conforms to the suggested structure (table 1). The FAB mass spectrum of (**1**) showed protonated molecular ion $[M^+ +1]$ at m/z 929. The molecule after the loss of C-17 side chain (CH_3CHOH) gave ion fragment at m/z 883 showing the point of attachment of the sugar chain to the only available C-3 hydroxyl group of the aglycon. The point of attachment of the sugar chain to the C-3 hydroxyl group of aglycon was further supported by the downfield shift of acetylated C-20 methine proton at δ5.11 with respect to the parent precursor in the ^{1}HNMR spectrum of penta-O-acetyl reticulin (**8**). The FABMS of (**1**) also showed the sugar component [tetrasaccharide + H] at m/z 613. The characteristic fragment ion[12] peaks supporting the sequence of sugars and the structure of (**1**) are discussed in experimental section. The structure of (**1**) was thus defined as

Table 1: ^{13}C NMR Shifts of (1), (2) and (3)

Carbon	(1)	(2)	(3)	Carbon	(1)		(2)		(3)
		Aglycone					Sugars		
1	36.1[a]	35.1	36.0	Cym-1	102.4	Dig-1	97.0	Cym-1	102.7
2	29.1[c]	29.6[a]	29.6[a]	2	36.4[a]	2	39.7	2	36.9[d]
3	75.8[d]	77.9	77.2	3	77.2	3	69.5	3	78.9
4	38.9[b]	38.7	38.8	4	85.2	4	81.8	4	82.8
5	140.7	140.4	140.8	5	70.9	5	67.5	5	68.2
6	121.1	121.5	120.2	6	18.6	6	18.2	OMe	56.1
7	29.1	29.6[a]	29.6[a]	OMe	60.0	Gal-1	103.3	Glu-1	103.9
8	33.3	32.7	32.7	Dig-1	99.3	2	75.7	2	73.9
9	53.0	56.1[b]	56.1[a]	2	39.3[b]	3	86.1	3	75.7[e]
10	38.4[a]	37.5[c]	37.5[d]	3	68.4	4	74.3	4	78.1
11	22.2	22.6	22.6	4	81.7	5	72.6	5	76.8[b]
12	36.4[a]	37.1[c]	37.1[d]	5	67.4	6	63.0	6	60.4
13	50.5	50.3	50.3	6	17.4[e]	OMe	60.6	Glu-1	104.2
14	87.1	87.0	87.0	Gal-1	103.7			2	72.4
15	31.3	31.9	31.9	2	75.9[d]			3	75.9[e]
16	28.7[c]	29.3[a]	29.3[a]	3	86.2			4	70.4
17	53.8	56.3[b]	56.3[c]	4	77.2			5	77.2[b]
18	13.6	14.0	14.0	5	71.4			6	60.4
19	15.7	16.4	16.4	6	63.1				
20	66.6	65.0	65.0	OMe	60.0				
21	19.2	19.8	19.8	Cym-1	103.0				
				2	38.4[b]				
				3	79.1				
				4	73.3				
				5	73.3				
				6	17.8[e]				
				OMe	57.6				

Cym = D-Cymarose, Dig = D-digitoxose, Gal = 3-O-methyl-D-galactose, Glu = D-glucose.

[a, b, c, d, e] Assignments within the column of each compound may be interchanged.

calogenin-3-O-β-D-cymaropyranosyl-(1→4)-O-3-O-methyl-α-D-galactopyranosyl-(1→4)-O-β-D-digitoxopyranosyl- (1→4) -O-β-D- cymaropyranoside.

Deniculatin (**2**), mp 124-127°C, $[\alpha]_D$ - 19.4°, $C_{34}H_{56}O_{11}$ (positive-ion FABMS: m/z 663 $[M^+ + Na]$) gave positive Liebermann- Burchardt, xanthydrol, Keller-Kiliani and Feigl tests[13] indicating it to be a steroidal glycoside of 2-deoxy and normal sugars. The ^{13}C NMR of (**2**) showed two anomeric carbons at δ 103.3 and 97.0 suggesting it to be a diglycoside. The ^1HNMR spectrum of (**2**) showed two anomeric proton signals at δ4.82 (1H, d, J=1 Hz) and δ 4.64 (1H, dd, J=9 and 2 Hz) confirming the diglycosidic nature of (**2**). The smaller coupling constant (J=1 Hz) for the normal sugar exhibiting anomeric doublet showed that it is glycosidically linked by an α-linkage in its $^4C_1$ (D) conformation, while the larger coupling (J=9 Hz) for the double-doublet suggested a β-glycosidic linkage for the 2- deoxy sugar in its $^4C_1$ (D) conformation. The ^1HNMR further showed methylene group signals in the region δ2.11-2.17 and 1.75-1.81 for the respective equatorial and axial protons of the 2-deoxy sugar alongwith two secondary methyl group doublets at δ1.22 (3H, d, J=7 Hz) and 1.19 (3H, d, J=6 Hz) and a

methoxy group singlet of three protons at δ3.49. The ^{13}C NMR data of (**2**) (table 1) was in close conformity with the results deduced from ^{1}H NMR spectrum.

Mild acid hydrolysis of (**2**) by Mannich and Siewert[8] method helped in the identification of the monosaccharides of glycoside (**2**). After two days the reaction mixture showed two spots, the less polar one was identical to calogenin (**4**) while the polar one was the glycon (**5**). Hydrolysis was complete in 5 days, showing three spots which on column chromatography afforded calogenin ($C_{21}H_{34}O_3$), D-digitoxose and 3-O-methyl-D-galactose. Thus in (**2**), 3-O-methyl-D-galactose is the terminal sugar attached to aglycon (**4**) through D-digitoxose.

The difference of $C_{13}H_{22}O_8$ between the molecular formulae of (**2**) and (**4**) further supported the presence of one normal and one 2,6- dideoxy hexose in (**2**). The FABMS showed [M^++Na] at m/z 663. The ion fragment at m/z 577 corresponding to [M^+-H_2O-(C17 side chain CH_3CHOH)] showed that the sugar chain is linked to the aglycon through C-3. In the EIMS of (**2**), the other fragments due to aglycon and sugar were recorded at m/z 289 and 306 corresponding to [genin - side chain] and [disaccharide - H_2O] respectively. It also contained the significant fragments of di- and monosaccharide units of (**2**). Compound (**2**) on acetylation with pyridine - Ac_2O yielded penta-O-acetyl deniculatin (**9**). The downfield shifting of H-20 of aglycone to δ4.56 in the ^{1}HNMR of (**9**) confirmed the attachment of the sugar chain to C-3 of the aglycon. Thus, the structure of (**2**) was established as calogenin-3-O-3-O-methyl-α-D-galactopyranosyl-(1→4)-O-β-D-digitoxopyranoside.

Leptaculatin (**3**), m.p. 107-110°C, $[\alpha]_D$-5.8° gave molecular formula $C_{40}H_{66}O_{16}$ supplemented by a protonated molecular ion peak at m/z 803 in the positive ion fast-atom- bombardment mass spectrum. The ^{13}C NMR of (**3**) which contains three anomeric carbons at δ104.2, 103.9 and 102.7 alongwith three anomeric protons at δ4.45 (1H) and 4.32 (2H) in conjunction with positive tests with Liebermann-Burchardt, xanthydrol and Keller-Kiliani reactions showed it to be steroidal triglycoside of deoxy sugar(s).

Compound (**3**) on hydrolysis with 0.05N H_2SO_4[9] in dioxan yielded calogenin (**4**) m.p. 199-201°C, $[\alpha]_D$-50° and a syrupy sugar $[\alpha]_D$-53.6° which was presumably the trisaccharide (**10**). To identify the monosaccharide units of (**3**), Mannich hydrolysis[8] was done. After 2 days, the reaction mixture exhibited two spots, the less polar one was identical in mobility with calogenin (**4**) while the polar one was the sugar (**10**). After 5 days two new spots were observed which were identified as D-cymarose and cellobiose (TLC, PC). After 7 days the hydrolysis was complete affording calogenin (**4**), D-cymarose and D-glucose.[14] For further characterization, D-cymarose and D-glucose were converted to their known D-cymaronic acid phenyl hydrazide[9] and D-gluconic acid phenyl hydrazide[14] respectively.

The configuration of the glycosidic linkages were ascertained by the ^{1}HNMR spectrum of (**3**) at 400 MHz. A two proton doublet (J=8 Hz) at δ4.32 in the spectrum was assigned to the two identical anomeric protons of two glucose units. A one proton double-doublet centered at δ4.45 (J=9 and 2 Hz) was attributed to the anomeric proton of the D-cymarose residue. The large coupling constants (8 and 9 Hz) were typical of an axial orientation of anomeric protons in the $^{4}C_1$ (D) conformation indicating β-glycosidic linkages for all the three sugar units. The structure of glycoside (**3**) was also supported by its ^{13}C NMR spectrum (table 1).

The FABMS of (3) also showed ion peak at m/z 757 which was attributed to [M^+-C17 side chain ($CHOHCH_3$)] confirming the point of attachment of the sugar chain to the only available C-3 hydroxyl group of the aglycon. In the ^1H NMR spectrum of octa-O- acetyl leptaculatin (11), the downfield shifting of acetylated methine proton at C-20 of the aglycon to δ4.67 with respect to its parent precursor in (3) further supported the point of attachment of sugar chain to the aglycon. Other significant fragments obtained were m/z 640 [M^+-(terminal Glu- OH)], 577 [640-H_2O-CH_3CHOH], 545 [577-CH_3OH], 486 [trisaccharide] and 469 [trisaccharide-OH] further confirming the derived structure. Consequently, the structure of (3) was deduced as calogenin-3-O-β-D-glucopyranosyl-(1→4)-O-β-D- glucopyranosyl-(1→4)-O-β-D-cymaropyranoside.

EXPERIMENTAL

General procedures were same as reported earlier.[9] ^1H and ^{13}C NMR spectra were recorded on a 400 MHz (Bruker) spectrometer in $CDCl_3$ with TMS as internal standard. FAB and EI mass spectra were recorded with JEOL mass spectrometer model JMS-SX102 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 (BDH) and column chromatography was done over silica-gel 60-120 mesh (Qualigens). Normal sugars were made visible by Partridge reagent on PC.

Plant extraction.

The aerial part of plant *L. reticulata* (30 kg) was collected locally. The identity of the plant was confirmed by Dr M.N. Srivastav, Botanist, Central Drug Research Institute, Lucknow, India, where a voucher specimen was deposited. Shade dried powdered plant material (10 kg) was extracted and fractionated by the method used for pregnane glycoside.[15] These fractions on evaporation yielded petroleum-ether, 1.2 g; Et_2O, 16.0 g; $CHCl_3$, 5.0 g; $CHCl_3$-EtOH (4:1), 2.3 g; $CHCl_3$-EtOH (3:2), 2.1 g. Repeated column chromatography of mixed $CHCl_3$ and $CHCl_3$-EtOH (4:1) extract using $CHCl_3$-MeOH (95:5) as eluent yielding Reticulin (1) (59 mg), Deniculatin (2) (50 mg) and Leptaculatin (3) (62 mg).

Reticulin (1), mp 119-122°C, $[\alpha]_D$-7.1° (c, 0.14; MeOH), (Found: C, 62.39; H, 8.61%; $C_{48}H_{80}O_{17}$ requires C, 62.07; H, 8.62%). It gave positive colours in xanthydrol, Keller-Kiliani and Liebermann-Burchardt tests. ^1H NMR δ0.75 (3H, s, 19Me), 1.0 (3H, s, 18Me), 1.27 (6H, d, J=7Hz, 21Me; 6'Me), 1.29 (3H, d, J=6 Hz, 6'Me), 1.32 (3H, d, J=6 Hz- 6'Me), 1.81-1.88 (3H, m, H-2'ax-S_1,S_2,S_4), 2.30-2.36 (3H, m, H-2' eq.- S_1, S_2, S_4), 3.46, 3.49, 3.52 (3H each, 3s, 3 x OMe), 3.55- 3.61 (5H, m, H-4'-S_1,S_2,S_3,S_4; H-2'-S_3), 4.26-4.36 (3H, m, H-6'-S_3; H-20), 4.73 (2H, dd, J=9 and 2 Hz; H-1'; S_1,S_4), 4.77 (1H, dd, J=8 and 2 Hz, H-1'-S_2), 5.16 (1H, d, J=4Hz, H-1'-S_3), 5.35 (1H, m, H-6). FABMS: m/z 929 [M^++1], 883 [M^+-CH_3CHOH], 851 [883-CH_3OH]$^+$, 833 [851-H_2O]$^+$, 789 [833-CH_3CHO]$^+$, 784 [M^+-(S_4-OH)]$^+$, 739 [789- CH_3OH-H_2O; 784-CH_3CHOH]$^+$, 721 [739 - H_2O]$^+$, 695 [739 - CH_3OH- CH_2OHCHO]$^+$, 671 [721-CH_3OH-H_2O]$^+$, 645 [721-CH_3OH- CH_3CHO]$^+$, 639 [671 - CH_3OH]$^+$, 633 [695 - H_2O - CH_3CHO]$^+$, 621 [639-H_2O]$^+$, 613 [tetrasaccharide +H]$^+$, 603 [695-CH_3OH-CH_2OHCHO]$^+$, 595 [tetrasaccharide-OH]$^+$, 583 [645-H_2O-CH_3CHO]$^+$, 577 [595-H_2O]$^+$, 573 [633-

$CH_2OHCHO]^+$, 559 [603-$CH_3CHO]^+$, 551 [583-$CH_3OH]^+$, 547 [583-$2H_2O]^+$, 545 [M^+-S_4-(S_3-OH)-CH_3CHOH-H_2O; 577-$CH_3OH]^+$, 543 [573-$2CH_3]^+$, 532 [547-$CH_3]^+$, 527 [545-$H_2O]^+$, 512 [527-$CH_3]^+$, 511 [tetrasaccharide-$C_5H_9O_2]^+$, 501 [545-$CH_3CHO]^+$, 497 [512-$CH_3]^+$, 493 [511-$H_2O]^+$, 491 [551-$CH_2OHCHO]^+$, 457 [501-$CH_3CHO]^+$, 451 [595-(S_4- OH)]$^+$, 443 [493-CH_3OH-$H_2O]^+$, 439 [457-$H_2O]^+$, 433 [M^+-S_4-S_3-(S_2-OH)-$CH_3CHOH]^+$, 429 [493-$2CH_3OH]^+$, 421 [453-$CH_3OH]^+$, 419 [451- $CH_3OH]^+$, 415 [433-$H_2O]^+$, 383 [415-CH_3OH; 443-$H_3COCHO]^+$, 379 [439- $CH_2OHCHO]^+$, 377 [421-$CH_3CHO]^+$, 369 [429-$CH_2OHCHO]^+$, 365 [457- CH_3OH-$CH_2OHCHO]^+$, 359 [419-$CH_2OHCHO]^+$, 347 [379-$CH_3OH]^+$, 339 [383-$CH_3CHO]^+$, 335 [$C_{15}H_{27}O_8]^+$, 333 [365-$CH_3OH]^+$, 327 [359- $CH_3OH]^+$, 325 [369-$CH_3CHO]^+$, 321 [M^+-(Genin-O-S_1-O-S_2)], 317 [335- $H_2O]^+$, 309 [327-$H_2O]^+$, 303 [335-$CH_3OH]^+$, 295 [339-$CH_3CHO]^+$, 289 [321-$CH_3OH]^+$, 281 [325-$CH_3CHO]^+$, 275 [M^+-Genin-S_4-(S_3-OH)], 273 [317-$CH_3CHO]^+$, 265 [309-$CH_3CHO]^+$, 257 [275-H_2O; 289-$CH_3OH]^+$, 243 [303-$H_3COCHO]^+$, 241 [273 - $CH_3OH]^+$, 213 [251-$CH_3CHO]^+$, 199 [243- $CH_3CHO]^+$, 197 [241-$CH_3CHO]^+$, 181 [213-$CH_3OH]^+$, 159 [$C_7H_{11}O_4]^+$, 115 [159-$CH_3CHO]^+$.

Deniculatin (2), mp 124-127°C, $[\alpha]_D$-19.4° (c, 0.155; MeOH), (Found: C, 63.6, H, 8.73%, $C_{34}H_{56}O_{11}$ requires C, 63.75; H, 8.75%). It responded positively to xanthydrol, Keller-Kiliani, Feigl and Liebermann-Burchardt reactions. ^{1}HNMR δ0.90 (3H, s, 19Me), 0.93 (3H, s, 18Me), 1.19 (3H, d, J=6Hz, 21Me), 1.22 (3H, d, J=7Hz, 6'Me -Dig), 1.75-1.81 (1H, H-2'ax-Dig), 2.11- 2.17 (1H, H2' eq-Dig), 3.49 (3H, s, OMe), 4.0-4.06 (1H, m, H-20), 4.64 (1H, dd, J=9 and 2Hz, H-1- Dig), 4.82 (1H, d, J=1Hz, H-1'- Gal), 5.28 (1H, m, H-6). FABMS m/z 663 [M^++Na], 622 [M^+-H_2O], 577 [622-$CH_3CHOH]^+$, 559 [577-$H_2O]^+$, 515 [559-$CH_3CHO]^+$, 509 [559-H_2O- $CH_3OH]^+$, 507 [M^+-$C_5H_9O_4$], 489 [507-$H_2O]^+$, 483 [515-$CH_3OH]^+$, 464 [M^+-(Gal-OH)], 429 [489-$CH_3OCHO]^+$, 419 [464-$CH_3CHOH]^+$, 408 [483- CH_3-$CH_2OHCHO]^+$, 404 [419-$CH_3]^+$, 393 [408-$CH_3]^+$, 386 [404-$H_2O]^+$, 371 [386-$CH_3]^+$, 369 [429-CH_3-$CH_2CHOH]^+$, 351 [369-$H_2O]^+$, 327 [371- $CH_3CHO]^+$, 307 [disaccharide-OH]$^+$, 289 [307-$H_2O]^+$, 211 [289-H_2O- $CH_2OHCHO]^+$. EIMS m/z 577 [M^+-CH_3CHOH-H_2O], 419 [M^+-(Gal-OH)- CH_3CHOH], 401 [577-(Gal-OH); 419-$H_2O]^+$, 386 [401-$CH_3]^+$, 371 [386- $CH_3]^+$, 368 [386-$H_2O]^+$, 339 [401-H_2O-$CH_3CHO]^+$, 306 [disaccharide- $H_2O]^+$, 289 [Genin-$CH_3CHOH]^+$, 256 [306-H_2O-CH_3OH; 289-H_2O-$CH_3]^+$, 238 [256-$H_2O]^+$, 228 [306-H_2O-$CH_2OHCHO]^+$, 214 [306-CH_3OH- $CH_2OHCHO]^+$, 131 [disaccharide-$C_5H_9O_4$-$H_3COCHO]^+$, 113 [131-$H_2O]^+$.

Leptaculatin (3), mp 107-110°C, $[\alpha]_D$-5.8° (c, 0.17; MeOH) (Found: C, 59.81; H, 8.25%; $C_{40}H_{66}O_{16}$ requires C, 59.85; H, 8.23%). It gave positive tests in the xanthydrol, Keller-Kiliani and Liebermann-Burchardt reactions. It underwent $NaIO_4$ oxidation. ^{1}H NMR δ0.88 (3H, s, 19Me), 1.0 (3H, s, 18Me), 1.25 (3H, d, J = 6Hz, 21Me), 1.29 (3H, d, J=6Hz, 6'Me-cym), 1.84-1.89 (1H, m, H-2'ax-cym), 2.28-2.34 (1H, m, H-2' eq-cym), 3.37-3.41 (1H, m, H-4'-terminal Glu), 3.52 (3H, s, OMe), 3.8 (1H, m, H-20), 4.32 (2H, d, J=8Hz, H-1'-Glu). 4.45 (1H, dd, J, 9 and 2Hz, H-1'-cym), 5.82-5.38 (1H, m, H-6). ^{13}C NMR data is given in Table 1. FABMS m/z 803 [M^++1], 784 [M^+-H_2O], 757 [M^+-CH_3CHOH], 742 [757-$CH_3]^+$, 739 [757-$H_2O]^+$, 724 [784-$CH_2OHCHO]^+$, 721 [739-$H_2O]^+$, 703 [721-$H_2O]^+$, 695 [742-CH_3-$CH_3OH]^+$, 651 [695-$CH_3CHO]^+$, 647 [724-CH_3CHOH-$CH_3OH]^+$, 640 [M^+-(terminal Glu-OH)], 635 [703-$2H_2O$-$CH_3OH]^+$, 603 [647-$CH_3CHO]^+$, 585 [603- $H_2O]^+$, 577 [640-H_2O-$CH_3CHOH]^+$, 573 [651-H_2O-$CH_2OHCHO]^+$, 555 [573- $H_2O]^+$, 549 [585-$2H_2O]^+$, 545 [577-$CH_3OH]^+$, 530 [545-$CH_3]^+$, 515 [530-$CH_3]^+$, 507 [585-H_2O-$CH_2OHCHO]^+$, 495 [555-

$CH_2OHCHO]^+$, 486 [trisaccharide]$^+$, 469 [trisaccharide-OH]$^+$, 437 [469-$CH_3OH]^+$, 419 [437-$H_2O]^+$, 401 [419-$H_2O]^+$, 391 [469-H_2O-$C_2H_4O_2]^+$, 383 [401- $H_2O]^+$, 365 [383-$H_2O]^+$, 355 [391-$2H_2O]^+$, 313 [391-H_2O-$CH_2OHCHO]^+$, 281 [313-$CH_3OH]^+$. EIMS m/z 460 [M^+ - terminal Glu - (Glu-OH)- H_2O], 450 [trisaccharide-$2H_2O]^+$, 436 [trisaccharide-CH_3OH-$H_2O]^+$, 433 [M^+-terminal Glu-(Glu-OH)-CH_3CHOH], 419 [M^+-terminalGlu-(Glu- OH)-$CH_3CHCHOH$-H], 418 [436-H_2O; 450-$CH_3OH]^+$, 404 [419-$CH_3]^+$, 401 [433-$CH_3OH]^+$, 400 [418-$H_2O]^+$, 386 [404-$H_2O]^+$, 383 [401-$H_2O]^+$, 382 [400-$H_2O]^+$, 357 [460-CH_3CHOH-$CH_3CHCHOH]^+$, 356 [357-H]$^+$, 353 [trisaccharide-$C_5H_9O_4]^+$, 342 [trisaccharide-cym]$^+$, 339 [383-$CH_3CHOH]^+$, 326 [386-CH_3-$CH_3CHOH]^+$, 325 [342-OH]$^+$, 324 [trisaccharide-(terminal Glu-OH)]$^+$, 321 [353-$CH_3OH]^+$, 317 [353- $2H_2O]^+$, 307 [325-H_2O; 324-OH]$^+$, 303 [321-$H_2O]^+$, 289 [307-$H_2O]^+$, 285 [303-$H_2O]^+$, 278 [324-OCHOH]$^+$, 277 [321-$CH_3CHO]^+$, 271 [289- H_2O; 317-HOCHO]$^+$, 264 [342-$C_2H_4O_2]^+$, 259 [277-$H_2O]^+$, 257 [289- $CH_3OH]^+$, 256 [324-CH_3OH-$2H_2O]^+$, 248 [324-CH_3OH-$CH_3CHO]^+$, 239 [257-H_2O; 285-HOCHO]$^+$, 231 [277-HOCHO]$^+$, 229 [289-$C_2H_4O_2]^+$, 228 [264-$2H_2O]^+$, 213 [259-HOCHO]$^+$, 210 [228-$H_2O]^+$, 209 [$C_7H_{13}O_7]^+$, 199 [259-$C_2H_4O_2]^+$, 197 [229-$CH_3OH]^+$, 191 [$C_8H_{15}O_5]^+$, 180 [Glu]$^+$, 163 [209-HOCHO; 180-OH]$^+$, 162 [cym]$^+$, 159 [191-$CH_3OH]^+$, 145 [cym- OH; 191-HOCHO; 163-$H_2O]^+$; 131 [191-$C_2H_4O_2]^+$, 127 [145-$H_2O]^+$, 115 [159-$CH_3CHO]^+$, 113 [145-$CH_3OH]^+$, 103 [$C_4H_7O_3]^+$, 95 [127-CH_3OH; 113-$H_2O]^+$, 87 [$C_4H_7O_2]^+$.

***Mannich Hydrolysis of* (1)**

To a solution of (**1**) (30 mg) in acetone (5 ml) conc. HCl (0.05 ml) was added. After 3 days, the reaction mixture showed a new spot identical to D-cymarose (TLC, PC). After 7 days, two more spots appeared which were identified as calogenin (**4**) and presumably a disaccharide (**5**) (TLC). Hydrolysis was complete after 10 days showing four spots identified as calogenin, D-cymarose, D- digitoxose and 3-O-methyl-D-galactose (TLC, PC, $[\alpha]_D$). Usual working followed by column chromatography gave calogenin (6.5 mg) mp 198- 200°C,$[\alpha]_D$ - 49.5° (c, 0.2, MeOH), D-cymarose (6.0 mg) $[\alpha]_D$ + 53.2° (c 0.125; H_2O), D- digitoxose (3.1 mg), $[\alpha]_D$ + 43.3° (c, 0.12, MeOH) and 3- O-methyl-D-galactose (3.4 mg), $[\alpha]_D$ + 165° (c, 0.15, H_2O).

***Very Mild Acid Hydrolysis of* (1)**

A solution of (**1**) (15 mg) in 1,4-dioxane (2.5 ml) was hydrolysed with 0.01 N H_2SO_4 (2.5 ml) at room temperature. After 10 days, reaction mixture exhibited three spots which were identified as D-cymarose (TLC), presumably (**6**) and unhydrolysed (**1**). After 15 days, two new spots (TLC) appeared which were probably of (**5**) and (**7**). The hydrolysis was complete after 20 days showing four spots which after usual work up gave (**4**) (3.4 mg) m.p. 201-203°C, D-cymarose (3.0 mg) $[\alpha]_D$ + 52.5° (c, 0.15, MeOH), D-digitoxose (1.4 mg) $[\alpha]_D$ + 42.5° (c 0.10, MeOH) and 3-O-methyl-D-galactose (1.7 mg) $[\alpha]_D$ + 165.5° (c 0.13, MeOH).

***Mannich Hydrolysis of* (2)**

To a solution of (**2**) (28 mg) in acetone (5 ml), conc. HCl (0.05 ml) was added. After 2 days the reaction mixture showed two spots identical with (**4**) and presumably the disachharide (**5**). After 5 days, hydrolysis was complete showing 3 spots identified as calogenin (**4**), D-digitoxose and 3-O-methyl-D-galactose (TLC, PC).

Usual work up followed by column chromatography yielded calogenin (8.2 mg) m.p. 200-202°C, D-digitoxose (3.6 mg) $[\alpha]_D$ + 42.9° (c 0.16, MeOH) and 3-O-methyl-D-galactose (4 mg) $[\alpha]_D$ + 166.0° (c 0.17, H_2O).

***Mild Acid hydrolysis of* (3).**

A solution of (3) (12 mg) in 80% aq 1;4-dioxane (1 ml) was hydrolysed with 0.1N H_2SO_4 (1 ml) at 50°C for 30 min. The usual work-up afforded genin (4) (4 mg) mp 199-201°C, $[\alpha]_D$-50° (c, 0.15, MeOH) and syrupy sugar (10) (5 mg) $[\alpha]_D$-53.6° (c, 0.2, MeOH).

***Mannich Hydrolysis of* (3).**

To a solution of (3) (30 mg) in acetone (5 ml) conc. HCl (0.05 ml) was added. After 2 days, the reaction mixture exhibited two spots identical to (4) and (10). After 5 days, two new spots were shown which were found identical with D-cymarose and cellobiose (TLC, PC). After 7 days the hydrolysis was complete showing three spots on TLC which were found identical with calogenin, D- cymarose and D-glucose. The usual work up afforded calogenin (7.8 mg) and two chromatographically pure sugars identified as D- glucose (7.5 mg) $[\alpha]_D$+51.3° (c 0.13, H_2O) and D-cymarose (4.1 mg) $[\alpha]_D$ + 51.4° (c 0.11, H_2O) with the authentic samples.

***Penta-O-Acetyl-Reticulin* (8)**

Compound (1) (3.5 mg) was dissolved in anhydrous 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 hr. Usual work up after the removal of excess of Ac_2O gave (8). ^{1}HNMR δ1.93-2.18 (15H, all s, together 5xOAc), 4.03-4.07 (1H, m, H-4, S_4), 5.08-5.14 (1H, m, H-20).

***Penta-O-Acetyl Deniculatin* (9)**

Glycoside (2) (4 mg) on acetylation with Ac_2O-C_5H_5N (1:1) at 100°C for 1 hr gave (9) after usual work up. ^{1}HNMR δ2.0- 2.15 (15H, all s, 5 x OAc), 3.62-3.65 (1H, m, H-4 Gal), 4.53-4.6 (1H, m, H-20).

***Octa-O-Acetyl Leptaculatin* (11)**

Glycoside (3) (4.2 mg) in C_5H_5N was mixed with Ac_2O (1:1) and the mixture was heated on a water bath at 100°C for 1 hr. Usual work up afforded (11). ^{1}HNMR δ1.98-2.12 (24H, all s, together 8 x OAc), 4.65-4.69 (1H, m, H-20).

D-Cymaronic Acid Phenyl Hydrazide.

Solutions of D-cymarose (4.0 and 3.5 mg), obtained from the hydrolysate of (1) and (3) respectively, in H_2O were oxidized with Br_2 using the usual method yielding syrupy lactones which on treatment with phenyl hydrazine yielded the known crystalline D- cymaronic acid phenyl hydrazides (1.5 and 0.9 mg) mp 151-153°C and 152-154°C.

D-Digitoxonic Acid Phenyl Hydrazide

Solutions of D-digitoxose (2.8 and 3.0 mg), obtained as hydrolysis products from (1) and (2) respectively, in H_2O were oxidized with Br_2 yielding syrupy lactones. These lactones on reaction with phenyl hydrazine yielded known crystalline D- digitoxonic acid phenyl hydrazide (1.0 and 1.2 mg) mp 121-122°C and 121-123°C.

D-Gluconic Acid Phenyl Hydrazide

Solution of D-glucose (4.0 mg), from hydrolysate of (**3**), in H_2O was oxidised with Br_2 using the usual method yielding lactones which on further treatment with phenyl hydrazine gave D-gluconic acid phenyl hydrazide (1.6 mg) mp 195-197° C.

Methyl-3-O-methyl-α-D-galactopyranoside

3-O-methyl-D-galactose (3.0 and 3.5 mgs) obtained from the hydrolysis of (**1**) and (**2**), were refluxed with absolute MeOH at 70°C for 18 hrs in the presence of cation exchange IR (120) H^+ resin. The reaction mixture was filtered while hot and filtrate was concentrated. Column chromatography of the concentrates gave methyl-3-O-methyl-α-D-galactopyranoside (1.2 and 1.8 mg) $[\alpha]_D$ + 161.9° (c, 0.1, MeOH) and +162.4° (c, 0.12, MeOH)

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REFERENCES

1. Kaneda, N.; Chai, H.; Pezzuto, J.M.; Kinghorn, A.D.; Farnsworth, N.R.; Tuchinda, P.; Udchachon, J.; Santisuk, T.; Rentrakul, V. *Planta Med.* **1992**, *58*, 429-431.
2. Mitsuhashi, H.; Hayashi, K. *Shoyaku.Zasshi* **1985**, *39*, 1-27.
3. Deepak, D.; Khare, A.; Khare, M.P. *Phytochemistry* **1989**, *28*, 3255-3263.
4. Satyavati, G.V.; Gupta, A.K.; Tandon, N.; Seth, S.D. Eds. *Medicinal Plants of India (Vol.2)*, Indian Council of Medical Research: N. Delhi **1987**, pp. 151-160.
5. Sastry, B.S.; Vijaylakshmi, T.; Rao, D.V.; Rao, E.V. *Indian Drugs* **1985**, *22*, 611-612.
6. Krishna, P.V.G.; Rao, E.V.; Rao, D.V. *Planta Med.* **1975**, *27*, 395-400.
7. Prakash, K.; Sethi, A.; Deepak, D.; Khare, A.; Khare, M.P. *Phytochemistry* **1991**, *30*, 297-299.
8. Srivastava, S.; Khare, M.P.; Khare, A. *Phytochemistry* **1993**, *32*, 1019-1021.
9. Khare, N.K.; Khare, M.P.; Khare, A. *Phytochemistry* **1984**, *23*, 2931-2935.
10. Collins, P.M., Ed. *Carbohydrates*, Chapman and Hall: London, **1987**; pp. 360.
11. Allgeier, H. *Helv. Chim. Acta* **1968**, *51*, 311-325.
12. Khare, M.P.; Khare, A. *J. Carb. Chem.* **1987**, *6*, 523-535.
13. Feigl, F. *Spot Tests in Organic Analysis*. 7th Edn, Elsevier: Amsterdam, **1975**; pp. 337.
14. Collins, P.M. Ed. *Carbohydrates*, Chapman and Hall: London, **1987**, pp. 244-245.
15. Shaub, F.; Kaufmann, H.; Stöcklin, W.; Reichstein, T. *Helv. Chim Acta.* **1968**, *51*, 738-767.

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