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PREGNANE GLYCOSIDES FROM *HEMIDESMUS INDICUS*

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Key Word Index—*Hemidesmus indicus*; Asclepiadaceae; pregnane glycosides; medidesmine; hemisine; desmisine.

Abstract—Three new pregnane oligoglycosides, medidesmine, hemisine and desmisine isolated from the plant *Hemidesmus indicus* were identified as sarcostin-3-*O*-α-D-glucopyranosyl (1→4)-*O*-β-D-digitoxopyranosyl (1→4)-*O*-β-D-oleandropyranoside, calogenin-3-*O*-β-D-cymaropyranosyl (1→4)-*O*[3-*O*-methyl]β-D-glucopyranosyl (1→4)-*O*-β-D-glucopyranosyl (1→4)-*O*-β-D-cymaropyranoside and calogenin-3-*O*-β-D-xylopyranosyl (1→4)-*O*-β-D-digitoxopyranosyl (1→4)-*O*-β-D-xylopyranosyl (1→4)-*O*-β-D-digitoxopyranoside, respectively, with the help of FAB-MS, EI-MS, ^{1}H and ^{13}C NMR spectroscopy, along with chemical transformations. Copyright © 1996 Published by Elsevier Science Ltd

INTRODUCTION

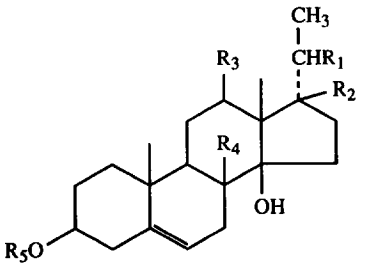
The plants belonging to the Asclepiadaceae family are reported to be rich in pregnane and cardiac glycosides [1, 2]. In recent years, the pregnanes and their glycosides have been shown to possess antitumour and anticancer activities [3, 4]. The plant *Hemidesmus indicus* (R.Br.) has been reported to be used against syphilis, chronic rheumatism, urinary diseases and skin infection in folk remedies [5]. In order to obtain biologically active pregnane glycosides from Asclepiadaceae plants five glycosides, desinine [6], indicine, hemidine [7], hemidescine, and emidine [8], were reported earlier from the chloroform and the chloroform–ethanol (4:1) extracts of *H. indicus*. In continuation of earlier work we now report the structure of three novel pregnane oligoglycosides **1**, **2** and **3** isolated from the chloroform–ethanol (3:2) extract of *H. indicus*.

RESULTS AND DISCUSSION

Medidesmine (**1**) mp 116–118°, $[\alpha]_D$ −27.6°C, $C_{40}H_{66}O_{17}$ responded positively to the Liebermann–Burchardt [9], xanthydrol [10], Keller–Killiani [11], and Feigl [12] tests, indicating it to be a steroidal glycoside of normal and 2,6-dideoxy sugar(s). The presence of signals for three anomeric protons and three anomeric carbons at δ 5.08, 4.52, 4.36 and δ 106.7, 101.9, 97.0 in its ^{1}H and ^{13}C NMR spectra, respectively, suggested it to be a triglycoside.

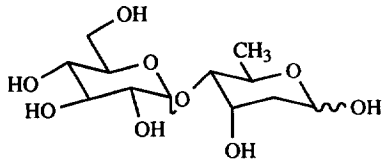
To identify the genin, the sugars, and their sequence in **1**, the compound was hydrolysed by the method of Mannich and Siewert [13] (with TLC and PC monitoring). After 6 days, the spot of starting material remaining in the reaction mixture was accompanied by two new spots which were presumably a disaccharide (**4**) and a monoglycoside (**5**). After 10 days, two additional spots identical in mobilities with oleandrose and sarcostin (**6**) appeared leading to the conclusion that oleandrose was linked to sarcostin. After 14 days, the spot of disaccharide (**4**) disappeared with the simultaneous appearance of two more spots identical in mobilities with glucose and digitoxose, indicating that the disaccharide was made up of glucose and digitoxose; digitoxose being a deoxy sugar was linked to oleandrose. The hydrolysis was complete in 17 days affording aglycone (6) which was identified as sarcostin [14] by comparison with the authentic sample (TLC, mmp, $[\alpha]_D$). Three chromatographically pure sugars were also obtained and identified as D-glucose [15], D-digitoxose [16] and D-oleandrose [17] by comparison with authentic samples ($[\alpha]_D$, PC, TLC). For further characterization D-glucose, D-digitoxose and D-oleandrose were oxidized with bromine water to give their respective lactones, which on treatment with phenylhydrazine yielded known crystalline D-gluconic acid phenyl hydrazide [15], D-digitoxonic acid phenyl hydrazide [16] and D-oleandronic acid phenyl hydrazide [17], respectively (mp, mmp).

The configurations of the glycosidic linkages were derived by the splitting patterns of anomeric proton signals in its ^{1}H NMR spectrum. A doublet at δ 5.08 (J = 1.5 Hz) and two double doublets at 4.52 (J = 8 and 2 Hz) and 4.36 (J = 8 and 2 Hz) were assigned to the anomeric protons of the three sugars. The small coupling constant of the doublet was typical of the equatorial orientation of an anomeric proton in a hexopyranose moiety in the $^4C_1$ (D) conformation suggesting that glucose was linked to digitoxose



1 $R_1 = R_2 = R_3 = R_4 = OH$, $R_5 = R_6$ (R = H)
2 $R_1 = OH$, $R_2 = R_3 = R_4 = H$, $R_5 = R_8$
3 $R_1 = OH$, $R_2 = R_3 = R_4 = H$, $R_5 = R_{10}$
5 $R_1 = R_2 = R_3 = R_4 = OH$, $R_5 = R_7$
6 $R_1 = R_2 = R_3 = R_4 = OH$, $R_5 = H$
7 $R_1 = R_3 = OAc$, $R_2 = R_4 = OH$, $R_5 = R_6$, R = Ac
8 $R_1 = OH$, $R_2 = R_3 = R_4 = H$, $R_5 = R_9$
9 $R_1 = OH$, $R_2 = R_3 = R_4 = R_5 = H$
12 $R_1 = OH$, $R_2 = R_3 = R_4 = H$, $R_5 = R_{11}$

4



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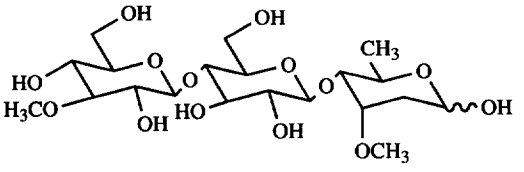

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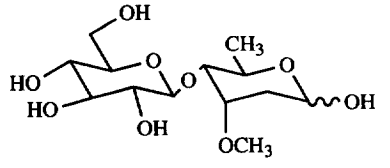

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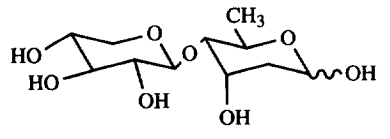
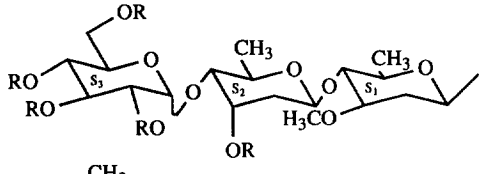
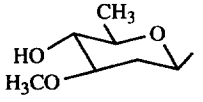


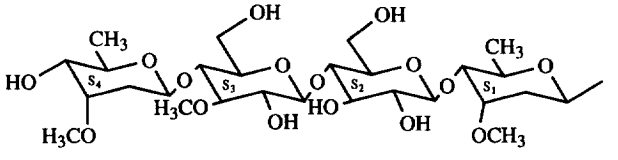
$R_6 =$



$R_7 =$



$R_8 =$



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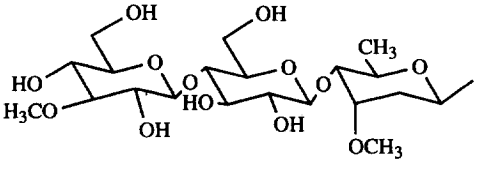
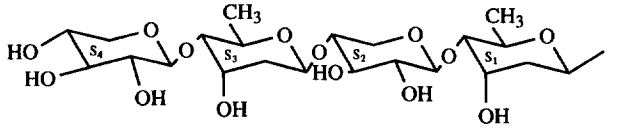
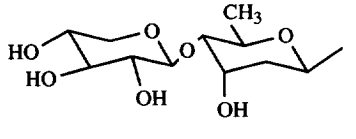


$R_{10} =$



$R_{11} =$



through an α-glycosidic linkage while the large coupling constant of the double doublets was typical of the axial configuration of the anomeric proton in a hexopyranose moiety in the $^4C_1$ (D) conformation indicating that these 2-deoxy sugars were linked through β-glycosidic linkages [18].

The FAB-mass spectrum of **1** did not record the $[M]^+$ ion but the highest mass ion peak was at m/z 803 and corresponded to $[M - CH_3]^+$. The other important signals at m/z 382 and 455 were due to $[genin]^+$ and $[trisaccharide + 1]^+$. The FAB-mass spectrum also confirmed the position of the sugar–genin linkage. The ion peak at m/z 737 arose from the loss of the C-17 side chain $[M - CH_3CHOH - 2H_2O]^+$ and m/z 615

resulted from the loss of the C and D rings from $[M]^+$ thus confirming the point of attachment of the sugar chain to the C-3 hydroxyl group of the aglycone. The sequence of sugars in **1** was also supported by the mass spectral studies. The ion peaks at *m/z* 611 $[M - Glu - CH_2CHOH]^+$ and 527 $[M - Glu - Dig + 1]^+$ further confirmed that the sequence of sugars in **1** was glucose–digitoxose–oleandrose. Structurally significant lower mass ion fragments were recorded in the EI-mass spectrum of **1**.

The positions of inter- and intra-glycosidic linkages were confirmed by the ^1H NMR studies of its hepta-*O*-acetyl derivative **7**. The signals for H-12 and H-20 appeared at δ 4.66 and 5.04 which were shifted downfield with respect to the H-12 and H-20 in **1** indicating that C-12 and C-20 hydroxyl groups were free, and the sugar chain was glycosidically linked to the remaining secondary hydroxyl group at C-3 of sarcostin. The $(1 \rightarrow 4)$ linkage between S_2 and S_3 was confirmed by the downfield shift of H-3′ of S_2 in the ^1H NMR of **7**. The ^{13}C NMR spectrum confirmed the deduced structure and also provided information about the position of the sugar–sugar and sugar–genin linkages. The presence of C-4′ signals of oleandrose and digitoxose at δ 83.2 and 83.9 indicated that the linkage between glucose–digitoxose and digitoxose–oleandrose was $1 \rightarrow 4$. The glycosidation shift of the aglycone carbon signals was observed at the C-2, C-3 and C-4 position confirming the attachment of the sugar chain at the C-3 hydroxyl group of the aglycone.

In view of the foregoing evidence the structure of **1** was established as sarcostin-3-*O*-α-D-glucopyranosyl $(1 \rightarrow 4)$-O-β-D-digitoxopyranosyl $(1 \rightarrow 4)$-*O*-β-D-oleandropyranoside.

Hemisine (**2**) mp 128–130°, $[\alpha]_D - 52.5°$, $C_{48}H_{80}O_{19}$ gave positive Liebermann–Burchardt [9], xanthydrol [10] and Feigl [15] tests, suggesting it to be a steroidal glycoside of 2,6-dideoxy and normal sugar(s). The presence of four anomeric protons at δ 4.58, 4.45, 4.37 and 4.32 in the ^1H NMR spectrum of **2** and four anomeric carbon signals at δ 102.9, 102.0, 99.4 and 97.8 in its ^{13}C NMR spectrum suggested it to be a tetraglycoside.

To identify the genin and sugar(s) and their sequence, compound **2** was subjected to Mannich and Siewert [13] hydrolysis with TLC and PC monitoring. After two days, in addition to the unreacted **2**, two more spots appeared, one of which was identical in mobility with cymarose and the other was presumably a triglycoside (**8**), indicating that cymarose was the terminal sugar. After 3 days two additional spots appeared, one was identified as calogenin (**9**) and the other was probably a trisaccharide (**10**). After 7 days the spot of the trisaccharide disappeared leaving behind two new spots which were identified as 3-*O*-methyl-glucose and probably a disaccharide (**11**) thus indicating that 3-*O*-methyl-glucose was next in sequence after cymarose. After 10 days, one more spot appeared and it was identified as glucose suggesting that glucose was next in sequence. As no new 2-deoxy sugar appeared at this stage the fourth sugar unit in the sequence must be cymarose which was linked to calogenin. The hydrolysis was complete in 15 days yielding three chromatographically pure sugars and an aglycone which were identified as D-cymarose [19], 3-*O*-methyl-D-glucose, D-glucose [15] and calogenin [21], respectively, by comparison with authentic samples ($[\alpha]_D$, mmp TLC, PC). D-Cymarose and D-glucose were further characterized by preparing their respective acid phenyl hydrazide. 3-*O*-Methyl glucose was converted to methyl-3-*O*-methyl-α-D-glucopyranoside [20] and compared with its authentic sample ($[\alpha]_D$, mmp TLC, PC).

The ^1H NMR spectrum of **2** further confirmed the derived structure and helped in ascertaining the configuration of the glycosidic linkages. Two doublets at δ 4.58 ($J = 8$ Hz), 4.37 ($J = 9$ Hz) and two double doublets at δ 4.45 ($J = 8$ and 2 Hz), 4.32 ($J = 8$ and 1.5 Hz) were assigned to the anomeric protons of the four sugars. The large coupling constants of these anomeric protons were typical of their axial configuration in a hexopyranose $^4C_1$ (D) [18] conformation suggesting that these sugar moieties were joined through β-glycosidic linkages.

The FAB-mass spectrum of **2** did not exhibit an $[M]^+$ ion, but the highest mass ion peak recorded at *m/z* 915 originated from the loss of the C-17 side chain from $[M]^+$, indicating that the sugar chain was glycosidically linked to the C-3 hydroxyl group of calogenin. The ion peaks at *m/z* 780, 640 and 479 were due to $[M - \text{cymarose} - 2H_2O]^+$, $[M - \text{cymarose} - (3\text{-}O\text{-methyl-glucose})]^+$ and $[M - \text{cymarose} - (3\text{-}O\text{-methyl-glucose}) - \text{glucose} + 1]^+$, respectively, and thus further confirming the sequence of sugars as derived by acid hydrolysis. The ion peaks at *m/z* 644 and 316 corresponded to $[\text{tetrasaccharide}]^+$ and $[\text{calogenin} - H_2O]^+$, respectively.

In the EI-mass spectrum of **2** the ion peaks at *m/z* 334, 194, 180 and 162 corresponded to $[\text{calogenin}]^+$, $[3\text{-}O\text{-Me-glucose}]^+$, $[\text{glucose}]^+$ and $[\text{cymarose}]^+$, respectively. The ^{13}C NMR chemical shifts of **2** are shown in Table 1. The glycosidation shifts of the aglycone carbons were observed at C-2, C-3 and C-4, hence the sugar moiety was linked to the C-3 hydroxy group of the aglycone. Among the carbon signals due to the sugar moiety the C-4′ of glucose, 3-*O*-methyl-glucose and one cymarose were found shifted downfield indicating a $1 \rightarrow 4$ glycosidic linkage between the sugars.

In light of the foregoing evidence the structure of hemisine was established as calogenin-3-*O*-β-D-cymaropyranosyl $(1 \rightarrow 4)$-*O*-(3-*O*-methyl)-β-D-glucopyranosyl $(1 \rightarrow 4)$-*O*-β-D-glucopyranosyl $(1 \rightarrow 4)$-*O*-β-D-cymaropyranoside.

Desmisine (**3**), mp 98–100°, $[\alpha]_D + 205.3°$ $C_{43}H_{70}O_{17}$, gave positive Liebermann–Burchardt [9], xanthydrol [10] and Keller–Killiani [11] tests suggesting it to be a steroidal glycoside of 2,6-dideoxy sugar(s). The presence of four anomeric proton signals at δ 4.80 (1H), 4.68 (1H) and 4.50 (2H) in the ^1H NMR spectrum of **3** suggested that **3** was a tetra-

Table 1. ^{13}C NMR shifts of compounds **1–3**

Aglycone				Sugars					
C	**1**	**2**	**3**	C	**1**	C	**2**	C	**3**
1	40.4	37.5	38.4	Dig-1	97.0	Glu-1	102.9	Dig-1	96.2
2	30.3	28.1	29.2	2	38.7	2	74.8	2	39.2
3	77.4	77.3	77.4	3	67.3	3	77.8	3	69.9
4	39.5	39.8	40.2	4	83.9	4	79.8	4	83.5
5	141.8	148.3	148.4	5	67.3	5	78.8	5	67.8
6	122.5	126.0	123.3	6	18.8	6	62.5	6	18.0
7	32.5	29.8	28.9	Glu-1	106.7	3MeGlu-1	102.2	Dig-1	99.8
8	75.3	34.0	32.2	2	75.3	2	75.2	2	39.5
9	45.8	48.5	49.8	3	77.3	3	85.8	3	70.6
10	37.9	37.7	36.8	4	72.1	4	80.6	4	83.5
11	24.4	24.4	24.7	5	78.6	5	79.0	5	67.9
12	71.5	36.8	38.4	6	63.1	6	62.5	6	18.8
13	56.0	45.0	46.7	Ole-1	101.9	OMe	61.3	Xyl-1	101.0
14	84.9	83.0	84.6	2	37.4	Cym-1	97.8	2	74.2
15	32.5	30.5	31.4	3	80.0	2	35.8	3	77.2
16	31.7	28.1	27.5	4	83.2	3	77.3	4	81.0
17	87.2	52.7	50.6	5	72.8	4	83.0	5	65.4
18	12.2	12.6	13.4	6	18.0	5	68.8	Xyl-1	102.0
19	18.8	17.8	17.6	OMe	57.3	6	18.5	2	75.4
20	71.5	70.2	71.7			OMe	55.2	3	77.3
21	15.5	22.8	23.5			Cym-1	99.4	4	70.8
						2	36.0	5	67.8
						3	77.0		
						4	73.8		
						5	69.3		
						6	18.8		
						OMe	57.5		

Value (ppm) from internal TMS in $CDCl_3$.

Ole: D-oleandrose, Cym: D-cymarose, Xyl: D-xylose, Dig: D-digitoxose, Glu: D-glucose, 3MeGlu: 3-*O*-methyl D-glucose.

glycoside. The tetraglycosidic nature of **3** was further confirmed from the presence of four anomeric carbon signals at δ 102.0, 101.0, 99.8 and 96.2 in its ^{13}C NMR spectrum.

To identify the genin and sugars of **3**, and to determine their sequence, it was subjected to Mannich and Siewert [13] hydrolysis. After two days, in addition to the spot of unreacted **3**, the hydrolysate exhibited two spots which were presumably a diglycoside (**12**) and a disaccharide (**13**). After 3 days, only two spots were left which were identified as calogenin (**9**) and disaccharide (**13**) indicating that **3** was made up of two similar disaccharide units. The hydrolysis was complete in 8 days yielding the spots of the aglycone and two chromatographically pure sugars identified as D-digitoxose [16], D-xylose [22] by comparison with the authentic samples ($[\alpha]_D$, PC, TLC). The aglycone was identified as calogenin [21] by direct comparison with the authentic sample (mmp, $[\alpha]_D$, TLC). The result of this hydrolysis indicated that the disaccharide was made up of digitoxose and xylose and the sequence of sugars in **3** was xylose–digitoxose–xylose–digitoxose and digitoxose being the deoxy sugar was linked to calogenin. The characterization of D-digitoxose and D-xylose was further supported by preparing their D-digitoxonic acid phenyl hydrazide [16] and methyl-*O*-β-D-xyloside [23], respectively, and comparing them with their respective authentic samples (mmp, TLC).

The configurations of the glycosidic linkages in **3** were confirmed from the ^1H NMR spectrum at 400 MHz. Two double doublets at δ 4.80 (1H, $J = 8$ and 2 Hz), 4.68 (1H, J = 8 and 2 Hz) and a doublet at δ 4.50 (2H, $J = 8$ Hz) were assigned to the anomeric protons of the four sugars. The large coupling constants of these anomeric protons were typical of their axial configurations in a hexopyranose moiety in the $^4C_1$ (D) conformation suggesting that these sugars were linked through β-glycosidic linkages [18].

The FAB-mass spectrum of **3** did not exhibit a molecular ion peak but the highest mass ion peak was recorded at *m/z* 843 corresponding to $[M - CH_3]^+$. The ion peak at *m/z* 762 was due to $[843 - CH_3CHOH - 2H_2O]^+$ which indicated that the C-20 hydroxyl group was free and the sugar chain was linked at the C-3 hydroxyl group of calogenin. The ion peaks at *m/z* 543, 334 were due to [tetrasaccharide + 1]$^+$ and [calogenin]$^+$. Further substantiation of the sugar sequence was obtained from the FAB-mass spectrum of **3**, which recorded fragment ions at *m/z* 678 $[M - xyl - H_2O - HCHO]^+$, 581 $[M - Xyl - Dig - CH_3]^+$ and 464 $[M - Xyl - Dig - Xyl]^+$ confirming the sugar sequence as derived from acid hydrolysis.

In the ^{13}C NMR spectrum of **3** the sugar carbon signals were assignable to two β-linked digitoxopyranose moieties and two β-linked xylopyranose units. The C-4′ signals of both digitoxose molecules and of one

xylose molecule were shifted downfield indicating that these sugars were glycosylated at the C-4 hydroxyl group (i.e. $1\rightarrow4$ glycosidic linkage). Glycosidation shifts of the aglycone carbon signals were observed at the C-2, C-3 and C-4 positions confirming the points of attachment of the sugar chain to the aglycone at its C-3 hydroxyl group.

In the light of the foregoing evidence the structure of desmisine was established as calogenin-3-*O*-β-D-xylopyranosyl $(1\rightarrow4)$-*O*-β-D-digitoxopyranosyl $(1\rightarrow4)$-*O*-β-D-xylopyranosyl $(1\rightarrow4)$-*O*-β-D-digitoxopyranoside.

EXPERIMENTAL

General procedures were the same as reported earlier [24]. ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz (Bruker) spectrometer in $CDCl_3$ with TMS as int. standard. FAB-MS, EI-MS were recorded with a JEOL mass spectrometer model JMS-SX 102 FAB with DA6000 Data system and D-300 with a IMA-2000 Data System, respectively. TLC was performed on silica-gel G (BDH) and CC was done over silica-gel 60–120 mesh (Qualigens). Normal sugars were made visible by Partridge reagent on PC.

Extraction. Shade-dried stems (6 kg) of *H. indicus* were extracted and fractionated with solvents of different polarities as reported earlier [6]. Repeated CC of the $CHCl_3$–EtOH (3:2) (2.4 gm) extract over silica gel using different polarities of $CHCl_3$–MeOH as eluent afforded medidesmine (**1**) (45 mg), hemisine (**2**) (44 mg) and desmisine (**3**) (52 mg).

Medidesmine (**1**). Mp 116–118°, $[\alpha]_D-27.6°$ (*c* 0.4, MeOH), found: C 58.54, H 8.08, $C_{40}H_{66}O_{17}$ requires C 58.68, H 8.06%. It gave a violet colour in the Liebermann–Burchardt test, a pink colour in xanthydrol, a blue colour in the Keller–Killiani test and a crimson colour in the Feigl test. For convenience the three monosaccharides of **1** were designated as S_1, S_2 and S_3 starting from the inner end. ^{1}H NMR: δ 5.36–5.30 (1H, *m*, H-6), 5.08 (1H, *d*, *J*=1.5 Hz, H-1′), 4.52 (1H, *dd*, *J*=8 and 2 Hz, H-1′), 4.36 (1H, *dd*, *J*=8 and 2 Hz, H-1′), 4.18–4.12 (1H, *m*, H-20), 4.08–4.00 (1H, *m*, H-6′, S_3), 3.90–3.82 (1H, *m*, H-6′, S_3), 3.80–3.70 (4H, *m*, H-5′, H-3′, S_1, S_2), 3.70–3.62 (1H, *m*, H-12), 3.60–3.54 (1H, *m*, H-5′, S_3), 3.38–3.32 (3H, *m*, H-2′, H-3′, H-4′, S_3), 3.30 (3H, *s*, OMe), 3.26–3.20 (2H, *m*, H-4′, S_1, S_2), 2.36–2.22 (2H, *m*, H-2′ eq, S_1, S_2), 2.02–1.90 (2H, *m*, H-2′ ax, S_1, S_2), 1.22 (3H, *s*, 18-Me), 1.14 (3H, *d*, *J*=6 Hz, 21-Me), 1.12 (3H, *d*, *J*=6 Hz, 6′ Me), 1.08 (3H, *d*, *J*=6 Hz, 6′ Me), 0.93 (3H, *s*, 19-Me). ^{13}C NMR data is given in Table 1. FAB-MS *m/z*: 803 $[M-Me]^+$, 737 $[M-CH_2CHOH-2H_2O]^+$, 722 $[803-MeCHOH-2H_2O]^+$, 704 $[722-H_2O]^+$, 677 $[737-MeOHCHO]^+$, 641 $[803-C_6H_{10}O_5]^+$, 615 $[M-C_{10}H_3O_4]^+$, 611 $(M-Glu-CH_2CHOH]^+$, 609 [641−MeOH], 601 $[677-MeOH-MeCHO]^+$, 583 $[601-H_2O]^+$, 565 $[609-CH_3CHO]^+$, 557 $[611-3H_2O]^+$, 539 $[583-MeCHO]^+$, 529 $[565-2H_2O]^+$, 527 $[M-Glu-Dig+1]^+$, 525 $[557-CH_2OH]^+$, 521 $[539-H_2O]^+$, 510 $[525-Me]^+$, 503 $[521-H_2O]^+$, 485 $[529-MeCHO]^+$, 481 $[525-MeCHOH]^+$, 467 $[485-H_2O]^+$, 463 $[485-H_2O]^+$, 455 [Trisaccharide+1]$^+$, 453 $[615-Glu]^+$, 445 $[463-H_2O]^+$, 435 $[453-H_2O]^+$, 430 $[445-Me]^+$, 421 $[453-MeOH]^+$, 419 $[454-OH-H_2O]^+$, 403 $[435-MeOH; 421-H_2O]^+$, 401 $[419-H_2O]^+$, 401 $[419-H_2O]^+$, 394 $[430-H_2O; 454-C_2H_4O_2]^+$, 382 $[M-sugar]^+$, 377 $[394-OH]^+$, 367 $[382-Me]^+$, 349 $[367-H_2O]^+$, 345 $[377-MeOH]^+$, 331 $[349-H_2O]^+$, 327 $[345-H_2O]^+$, 313 $[331-H_2O]^+$, 310 [Disaccharide]$^+$, 292 [Disaccharide]$^+$, 291 $[421-Dig]^+$, 277 $[313-H_2O]^+$, EI-MS *m/z* 293 $[Disaccharide-OH]^+$, 283 $[Genin-MeCHOH-3H_2O]^+$, 180 [Monosaccharide]$^+$, 163 $[180-OH]^+$, 162 [Monosaccharide]$^+$, 148 [Monosaccharide]$^+$.

Hemisine (**2**). Mp 128–130°, $[\alpha]_D-52.5°$ (*c*, 0.10, MeOH) found C 60.05, H, 8.30 $C_{48}H_{80}O_{19}$ requires C 60.00, H, 8.33%. It gave a violet colour in the Liebermann–Burchardt test, a blue colour in the Keller–Killiani test, a pink colour in xanthydrol and a crimson colour in the Fiegl test. For convenience the four monosaccharides of **2** were designated as S_1, S_2, S_3 and S_4 starting from the inner end. ^{1}H NMR: δ 5.36–5.00 (1H, *m*, H-6), 4.58 (1H, *d*, *J*=8 Hz, H-1′), 4.45 (1H, *dd*, *J*=8 and 2 Hz, H-1′), 4.37 (1H, *d*, *J*=9 Hz, H-1′), 4.32 (1H, *dd*, *J*=8 and 1.5 Hz, H-1′), 4.20–4.08 (2H, *m*, H-6′, S_2, S_3), 3.92–3.80 (2H, *m*, H-6′, S_2, S_3), 3.80–3.64 (4H, *m*, H-5′, H-3′, S_1, S_4), 3.64–3.58 (2H, *m*, H-5′, S_2, S_3), 3.55, 3.50, 3.48 (3H each, 3*S*, OMe, S_1, S_3, S_4), 3.41 (1H, *q*, *J*=8 Hz, H-20), 3.36–3.20 (8H, *m*, H-4′, S_1, S_4, H-2′, H-3′, H-4′, S_2, S_3), 2.14–2.02 (2H, *m*, H-2′ eq S_1, S_4), 2.00–1.84 (2H, *m*, H-2′ *ax*, S_1, S_4), 1.32 (3H, *d*, *J*=6 Hz, 21-Me), 1.22 (6H, *d*, *J*=6 Hz, 6′-Me, S_1, S_4), 0.97 (3H, *s*, 18-Me), 0.88 (3H, *s*, 19-Me), ^{13}C NMR data is given in Table 1. FAB-MS: *m/z* 915 $[M-MeCHOH]^+$, 883 $[915-MeOH]^+$, 851 $[883-MeOH]^+$, 791 $[851-CH_2OHCHO]^+$, 780 $[M-Cym-2H_2O]^+$, 720 $[780-CH_2OHCHO]^+$, 715 $[791-MeOH-MeCHO]^+$, 702 $[720-H_2O]^+$, 697 $[715-H_2O]^+$, 657 $[702-MeCHOH]^+$, 644 [Tetrasaccharide+1]$^+$, 642 $[702-CH_2OHCHO]^+$, 640 $[M-Cym-(3-O-Me-Glu)]^+$, 627 $[642-Me]^+$, 619 $[697-CH_2OHCHO]^+$, 608 $[640-MeOH; 644-2H_2O]^+$, 604 $[640-2H_2O]^+$, 601 $[619-H_2O]^+$, 544 $[604-CH_2OHCHO]^+$, 532 $[608-CH_2OH-MeCHO]^+$, 512 $[544-MeOH]^+$, 500 [Trisaccharide]$^+$, 479 $[M-Cym-(3-OMe-Glu)-Glu+1]^+$, 472 $[532-CH_2OHCHO]^+$, 467 $[512-MeCHOH]^+$, 455 $[472-OH]^+$, 423 $[467-MeCHO]^+$, 316 $[M-sugar-H_2O]^+$, 301 $[316-Me]^+$, 238 $[301-H_2O]^+$, EI-MS *m/z* 334 [Aglycon]$^+$, 317 $[334-OH]^+$, 302 $[317-Me]^+$, 194 [Monosaccharide]$^+$, 180 [Monosaccharide]$^+$, 162 [Monosaccharide; 194−MeOH]$^+$, 144 $[162-H_2O]^+$, 130 $[162-MeOH]^+$, 112 $[130-H_2O]^+$, 95 $[112-OH]^+$.

Desmisine (**3**). Mp 98–100°, $[\alpha]_D+205.3°$ (*c*, 0.10, MeOH) found C, 60.18, H, 8.14% $C_{43}H_{70}O_{17}$ requires C, 60.13; H, 8.15%. It gave a violet colour in the Liebermann–Burchardt test, a pink colour in xanthydrol

and a blue colour in the Keller–Killiani test. The four monosaccharides of **3** were designated as S_1, S_2, S_3 and S_4 starting from the inner end. ^{1}H NMR δ 5.38–5.30 (1H, *m*, H-6), 4.80 (1H, *dd*, J=8 and 2 Hz), 4.68 (1H, *dd*, J=8 and 2 Hz, H-1′), 4.50 (2H, *d*, J=8 Hz, H-1′), 3.98–3.82 (6H, *m*, H-5′, S_2, S_4, H-5′, H-3′, S_1, S_3), 3.64–3.58 (1H, *m*, H-20), 3.48–3.42 (2H, *m*, H-3′, S_2, S_4), 3.36–3.16 (4H, *m*, H-20), 3.48–3.42 (2H, *m*, H-3′, S_2, S_4), 3.36–3.16 (4H, *m*, H-4′, H-2′, S_2, S_4), 3.16–3.06 (2H, *m*, H-4′, S_1, S_3), 2.22–2.00 (2H, *m*, H-2′ *eq* S_1, S_3), 1.94–1.80 (2H, *m*, H-2′, *ax*, S_1, S_3), 1.36 (3H, *d*, J=6 Hz, 6′-Me), 1.26 (3H, *d*, J=6 Hz, 21-Me), 1.24 (3H, *d*, J=6 Hz, 6′-Me), 1.04 (3H, *s*, 18-Me), 0.92 (3H, *s*, 19-Me). ^{13}C NMR data is given in Table 1. FAB-MS: *m/z* 843 $[M-Me]^+$, 762 $[843-MeCHOH-2H_2O]^+$, 718 $[762-MeCHO]^+$, 678 $[M-Xyl-H_2O-HCHO]^+$, 618 $[678-Me-Me-CHOH]^+$, 581 $[M-Xyl-Dig-Me]^+$, 568 $[718-C_5H_8O_2-H_2O]^+$, 556 $[618-H_2O-MeCHO]^+$, 543 [Tetrasaccharide+1]$^+$, 536 $[586-MeCHOH]^+$, 536 $[581-MeCHOH]^+$, 506 $[568-MeCHO-H_2O$; 542 $-H_2O]^+$, 482 $[536-3H_2O]^+$, 464 $[M-Xyl-Dig-Xyl]^+$, 459 $[506-OH-HCHO]^+$, 456 $[718-C_{11}H_8O_7]^+$, 452 $[482-HCHO]^+$, 441 $[459-H_2O]^+$, 397 $[441-MeCHO]^+$, 379 $[397-H_2O]^+$, 344 $[379-HCHO]^+$, 334 [M−sugar], 316 $[334-H_2O]^+$, 299 $[316-OH]^+$, 289 $[334-MeCHOH]^+$, 253 $[289-2H_2O]^+$.

Mannich and Siewert hydrolysis of **1**. To a soln of **1** (25 mg)in Me_2CO (2.5 ml), conc. HCl (0.025 ml) was added at room temp. After 6 days two new spots, presumably a disaccharide (**4**) and a monoglycoside (**5**) appeared. After 10 days two additional new spots identified as cymarose and sarcostin (**6**) appeared. After 14 days the spot of the disaccharide disappeared leaving behind two new spots identified as glucose and digitoxose. Hydrolysis was complete in 17 days. Usual work-up afforded sarcostin (**6**) (3.9 mg) mp 260–263°, $[\alpha]_D$+62.88° (*c*, 0.12, MeOH] and three chromatographically pure sugars identified as D-glucose (3.4 mg) $[\alpha]_D$+52° (*c*, 0.15, H_2O), D-digitoxose (2.8 mg) $[\alpha]_D$+42.6° (*c*, 0.16, MeOH) and D-oleandrose (3.2 mg) $[\alpha]_D$−14.4° (*c*, 0.12, MeOH).

Mannich and Siewert hydrolysis of **2**. To a soln of compound **2** (20 mg) in Me_2CO (2.5 ml), conc. HCl (0.025 ml) was added at room temp. After 2 days the reaction mixt. exhibited two new spots identified as cymarose and presumably a triglycoside (**8**). After 3 days two new spots were appeared, identified as calogenin (**9**) and probably a trisaccharide (**10**). After 7 days the spot of trisaccharide disappeared leaving behind two new spots identified as 3-*O*-methyl-D-glucose and presumably a disaccharide. After 10 days, one more new spot, identified as D-glucose, appeared. The hydrolysis was complete in 15 days. Usual [13] work up afforded calogenin (**9**) (3.5 mg) mp 200–202° $[\alpha]_D$−49.6° (*c*, 0.11, MeOH), three chromatographically pure sugars identified as D-cymarose (2.8 mg) $[\alpha]_D$+49.9° (*c*, 0.11, H_2O), 3-*O*-methyl-D-glucose (3.2 (mg), $[\alpha]_D$+68.8° (*c*, 0.15, MeOH) and D-glucose (3.1 mg) $[\alpha]_D$+52.8° (*c*, 0.14, H_2O).

Acetylation of **1**. Compound **1** (5 mg) on acetylation with Ac_2O (1 ml) in pyridine (1 ml) at 100° for 4 hr and usual work-up afforded the heptaacetate (**7**). ^{1}H NMR: δ 5.08–5.01 (1H, *m*, H-20), 4.68–4.64 (1H, *m*, H-12), 4.62–4.58 (1H, *m*, H-3′, S_2), 2.10 (3H, *s*, OAc), 2.08 (6H, *s*, OAc), 2.06 (3H, *s*, OAc), 2.04 (6H, *s*, OAc), 2.02 (3H, *s*, OAc).

D-*Gluconic acid phenyl hydrazide*. Solns of D-glucose (3.0 and 1.9 mg) obtained from the hydrolysate of **1** and **2**, 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-gluconic acid phenyl hydrazide (1.8 and 0.8 mg), mp 194–195° and 195–197°.

D-*Digitoxonic acid phenyl hydrazide*. Solns of D-digitoxose (2.4 and 3.0 mg), obtained as hydrolysis products from **1** and **3**, respectively, were oxidized with Br_2 yielding syrupy lactones. These lactones on reaction with phenyl hydrazine yielded known crystalline D-digitoxonic acid phenyl hydrazide (1.1 and 1.5 mg) mp 120–121° and 120–122°.

D-*Oleandronic acid phenyl hydrazide*. A soln of D-oleandrose (2.5 mg) from the hydrolysate of **1** in H_2O was oxidized with Br_2 using the usual method yielding a lactone which on further treatment with phenyl hydrazine gave D-oleandronic acid phenyl hydrazide (1.4 mg) mp 132–134°.

D-*Cymaronic acid phenyl hydrazide*. A soln of D-cymarose (2.4 mg) obtained from the hydrolysate of **2** in H_2 was oxidized with Br_2 using the usual method yielding a lactone which on further treatment with phenyl hydrazine yielded D-cymaronic acid phenyl hydrazide (1.0 mg) mp 150–152°.

Methyl-3-O-methyl-α-D-glucopyranoside. 3-*O*-Methyl-D-glucose (2.6 mg) obtained from the hydrolysate of **2** was refluxed with absolute MeOH at 70° for 16 hr in the presence of cation exchange IR (120)H^+ resin. The reaction mixt. was filtered while hot and the filtrate was concd (1.2 mg) $[\alpha]_D$+147.5° (*c*, 0.1, H_2O).

Methyl-β-D-xylopyranoside. Xylose (4.5 mg) obtained from the hydrolysate of **3** was refluxed with absolute MeOH at 70° for 18 hr in the presence of cation exchange IR (120)H^+ resin. The reaction mixt. was filtered while hot and the filtrate was concd. CC of the concentrate gave methyl-β-D-xylopyranoside (2.6 mg) $[\alpha]_D$−64.9° (*c* 0.14, H_2O).

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