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Pregnane glycosides from *Hoodia gordonii*

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ABSTRACT

Hoodia gordonii is a 'weight loss' herb, which has gained popularity in the western countries as an appetite suppressant dietary supplement. Phytochemical study of its aerial parts led to isolation of seven pregnane glycosides (hoodigosides W–Z, hoodistanalosides A–B). Their structures were elucidated by chemical degradation studies and spectroscopic methods, including 1D and 2D NMR and CD spectroscopic methods.

1. Introduction

Hoodia gordonii (Asclepiadaceae) is a succulent plant, indigenous to the summer rainfall regions of the Kalahari Desert in South Africa, Namibia, and Botswana. There are 13 reported species of the *Hoodia* genus, which have been a part of African food for centuries (Anonymous, 2007; Muller and Albers, 2002). Various uses of *Hoodia* have also been reported in African folklore such as for treatment of abdominal cramps, hemorrhoids, tuberculosis, diabetes, and as an aphrodisiac (Folden, 2008, Rubin et al., 2002). However, it has recently been known as an appetite suppressant (Anonymous, 2007; MacLean and Luo, 2004; Van Heerden et al., 1998, 2007).

In our previous phytochemical studies on *H. gordonii*, we have reported the isolation and characterization of 11 new oxypregnane glycosides (hoodigosides A–K) (Pawar et al., 2007b), and 10 new calogenin glycosides (hoodigosides L–U) (Pawar et al., 2007a). These compounds were used as markers for developing HPLC, and LC–MS based methods for identification, and quality control of *H. gordonii* plant materials, and dietary supplements (Avula et al., 2006, 2007). Here we report the isolation, and characterization of seven new pregnane glycosides (**1**, **2**, **5–9**), along with three known compounds (**3**, **4**, **10**) (Schemes 2 and 3). These constituents were identified as the glycosides of hoodigogenin A, isoramanone, calogenin, and two novel chemotypes of aglycones, namely hoodistanal, and dehydrohoodistanal (Scheme 1). The structures of the isolates were identified by extensive spectroscopic and chemical studies.

2. Results and discussion

The $CHCl_3$ extract of the aerial parts of *H. gordonii* was fractionated by multistep column chromatography to yield steroidal glycosides **1–10**.

Based on the ^{13}C NMR spectrum and HRESIMS *m*/*z* 1045.5711 for a sodiated molecular ion, the molecular formula for compound **1** was determined as $C_{54}H_{86}O_{18}$. The presence of four sugar moieties was evident as its ^1H NMR spectrum showed signals for four anomeric protons at δ_H 4.72 (d, J = 7.2 Hz), 4.94 (d, J = 11.2 Hz), 5.12 (d, J = 9.6 Hz), and 5.26 (d, J = 9.2 Hz), and corresponding carbon resonances at δ_C 106.3, 100.8, 100.7, and 96.6, respectively. Upon acid hydrolysis, compound **1** yielded aglycone **1a**. Upon comparison of ^{13}C NMR and HR-MS characteristics of **1a** with the reported data (Pawar et al., 2007b), the structure of **1a** was confirmed as 12-*O*-β-tigloyl-3β,14β-dihydroxypregn-5-en-20-one (hoodigogenin A) (Scheme 1). The *E* configuration of the double bond in tigloyl(2-methyl-2-butenoic acid) substitution at C-12 was identified by the small coupling constant (J = 6.0 Hz) of the olefinic proton at C-3' (Table 1) (Pawar et al., 2007b). With the help

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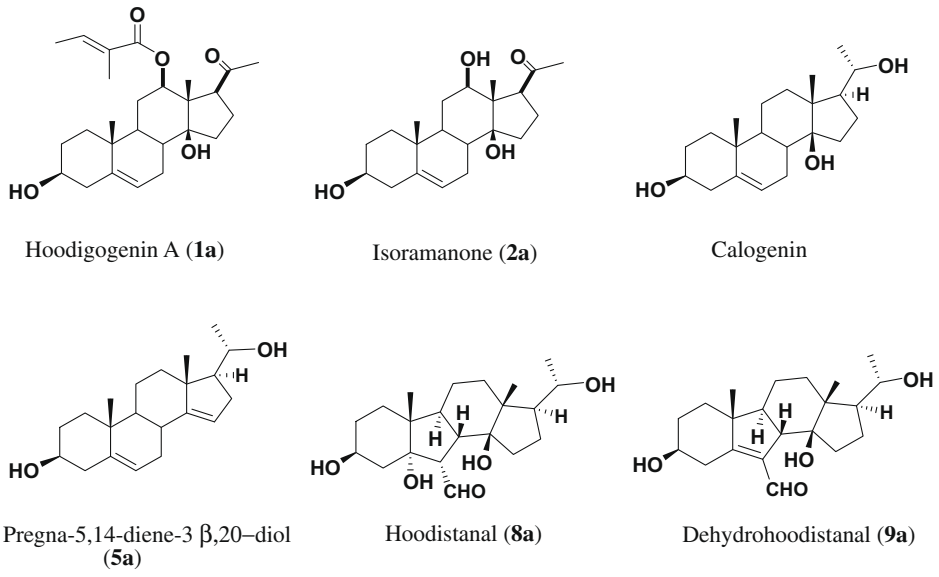


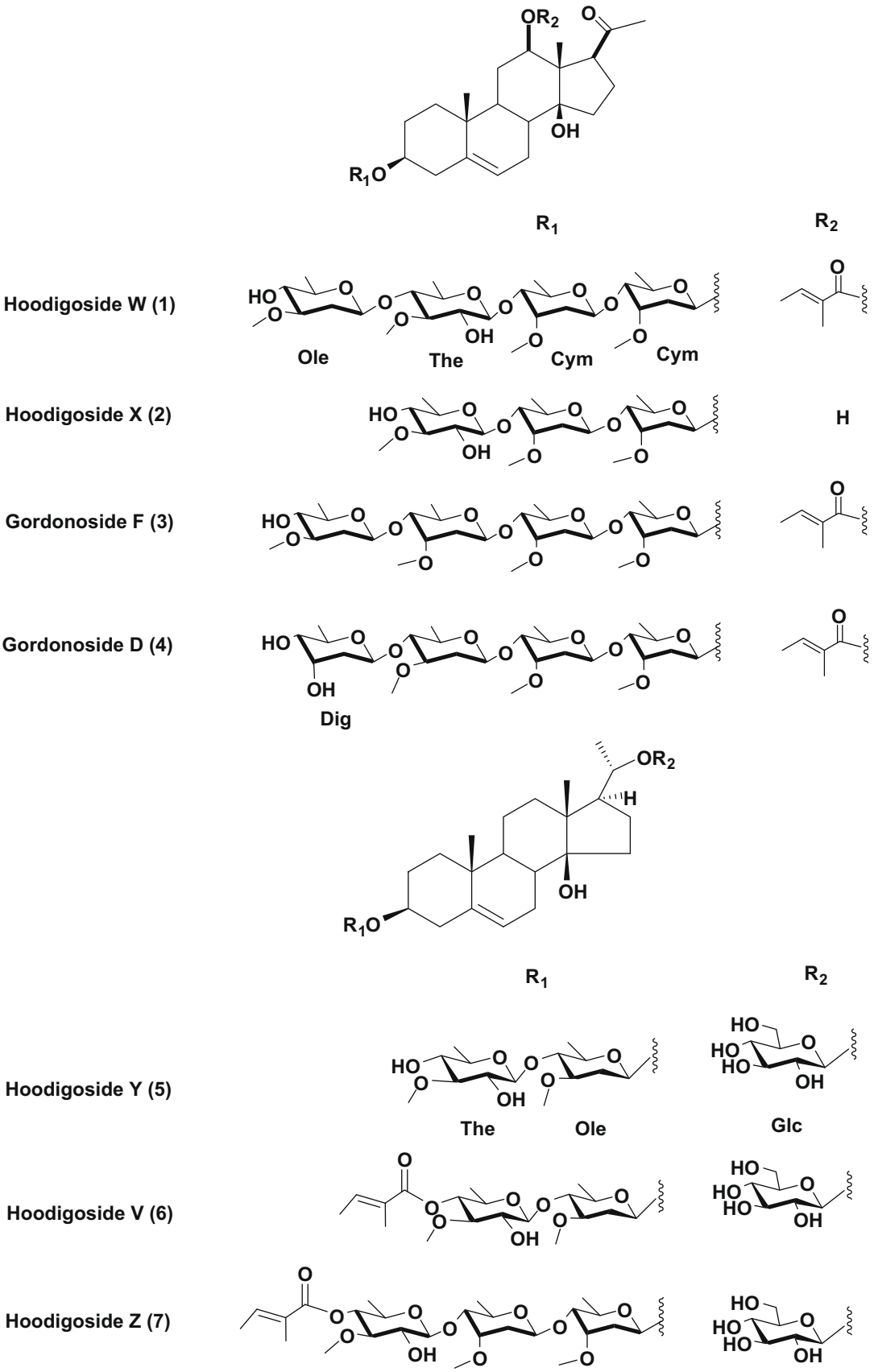
Scheme 1. Structures of aglycones for compounds **1**–**9** from *Hoodia gordonii*.

of 2D NMR (HMBC and COSY) spectroscopic data, the sugar units were identified as three 3-*O*-methyl-2,6-dideoxyhexopyranoses, and one 3-*O*-methyl-6-deoxyhexopyranose. These sugars were identified as D-cymarose, D-oleandrose, and D-thevetose via the hydrolysis studies. The ^{13}C NMR spectrum showed that C-1′ of the first cymarose unit resonated at δ_C 96.6, which is characteristic for a cymarose attached to C-3 (Table 2). This was further supported by the HMBC correlation of H-1′ (δ_H 5.26) with C-3 (δ_C 77.5). H-1″ (δ_H 5.12) of the second cymarose moiety showed long-range correlation with C-4′ (δ_C 83.7), and the anomeric proton of thevetose (δ_H 4.72) with C-4″ (δ_C 83.5). Further, H-4‴ of thevetose (δ_H/δ_C 3.54/82.7) showed HMBC correlation with C-1⁗ of oleandrose (δ_C 100.8). Thus, the sugar chain at C-3 was established as 3-*O*-β-D-oleandropyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside. Hence the structure of hoodigoside W (**1**) was established as 12-*O*-β-tigloyl-14β-hydroxypregn-5-en-20-one-3-*O*-β-D-oleandropyranosyl-(1→4)-β-D-thevetopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

The molecular formula of compound **2**, $C_{42}H_{68}O_{14}$, was deduced from the $[M+Na]^+$ ion at *m*/*z* 819.4571. The ^{13}C NMR data for **2** was generally similar to that of **1** except that the signals for the tigloyl moiety were absent. Upon acid hydrolysis, compound **2** produced aglycone **2a** (Scheme 1), which was identified as isoramanone (Warashina and Noro, 1998). ^1H NMR spectroscopic data of **2** established the presence of three sugars with their anomeric protons resonating at δ_H 4.25 (d, *J* = 7.6 Hz), 4.71 (d, *J* = 8.8 Hz), and 4.80 (d, *J* = 8.8 Hz). The corresponding carbons for these anomeric protons resonated at δ_C 104.3, 99.6, and 95.9, respectively (Table 2). Analysis of the acid hydrolysate identified two of these sugars as D-cymarose, and the third as D-thevetose. The anomeric proton of the first cymarose moiety (H-1′, δ_H 4.80) showed HMBC correlation with C-3 (δ_C 78.0) indicating attachment of the sugar moiety at C-3. Further, H-1″ (δ_H 4.71) of the second cymarose moiety showed a three-bond correlation to C-4′ (δ_C 82.5), and H-1‴ of the thevetose unit showed a correlation to C-4″ (δ_C 82.3). The C-4‴ of thevetose (δ_C 74.7) resonated upfield as compared to the corresponding signal in compound **1**, confirming that thevetose was the terminal sugar moiety of the saccharide sequence in compound **2**. Based on these observations, the structure of compound **2** was elucidated as 12β, 14β-dihydroxypregn-5-en-20-one-3-*O*-β-D-thevetopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside. Compound **2** was named hoodigoside X.

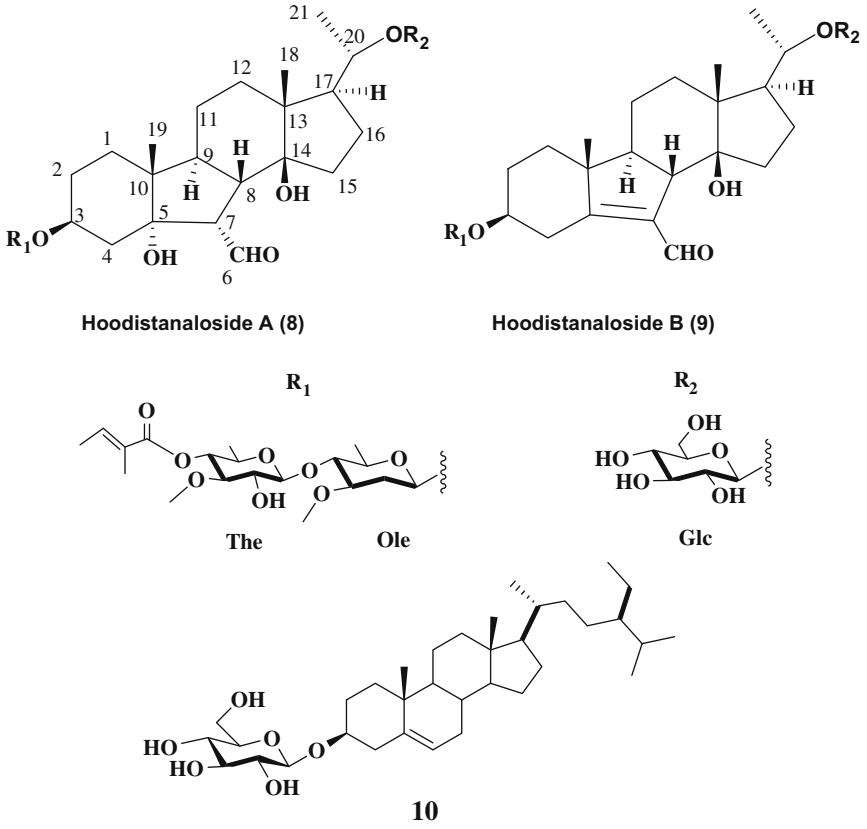
Based on positive HRESIMS data, compounds **3** and **4** had molecular formulae $C_{54}H_{86}O_{17}$ (*m*/*z* 1029.5761, $[M+Na]^+$) and $C_{53}H_{83}O_{17}$ (*m*/*z* 1015.5592, $[M+Na]^+$), respectively. The ^{13}C NMR spectra suggested that both of these compounds contained hoodigogenin A as aglycone. Further 1D and 2D NMR spectroscopic analyses facilitated establishment of the structures of **3** and **4** as gordonoside F and gordonoside D, respectively, which were previously reported from *H. gordonii* by Innocenti et al. (Dall'Acqua and Innocenti, 2007). The ^1H and ^{13}C NMR chemical shifts for **3** and **4** were consistent with the reported data.

Compound **5** was obtained as white amorphous powder. Its HRESIMS spectrum showed a quasi-molecular ion $[M+Na]^+$ at *m*/*z* 823.4519 that corresponded to a molecular formula $C_{41}H_{68}O_{15}$. The ^{13}C NMR spectrum displayed seven methyls, 20 methines, 10 methylenes, and four quaternary carbon atoms. The ^1H NMR signals for three anomeric protons were observed at δ_H 4.82 (d, *J* = 9.2 Hz), 4.87 (d, *J* = 7.6 Hz), and 4.94 (d, *J* = 7.6 Hz), with their corresponding carbons at δ_C 98.3, 104.4 and 105.2, respectively. The sugar moieties with anomeric protons at δ_H 4.82 and 4.87 were identified as 3-*O*-methyl-2,6-dideoxyhexopyranose and 3-*O*-methyl-6-deoxyhexopyranose, respectively, and the remaining one as a hexopyranose. These sugars were identified as oleandropyranose, thevetopyranose and glucopyranose by GC–MS of the acid hydrolysate as well as by examination of 2D NMR (HMQC, HMBC, COSY) data. The ^1H NMR signals for the aglycone portion of **5** suggested the presence of a Δ^5 pregnane as evidenced by a broad olefinic proton singlet at δ_H 5.54. The ^{13}C NMR spectrum indicated three oxygenated carbons at δ_C 77.9 (C-3), 84.3 (C-14), and 79.3 (C-20). To determine the skeleton of the aglycone, **5** was hydrolyzed with 0.05 N HCl to yield compound **5a**. The HRESIMS data of **5a** had an $[M+H]^+$ ion at *m*/*z* 317.2481 that was consistent with a molecular formula $C_{21}H_{32}O_2$. Based on this molecular formula, and detailed study of the NMR spectroscopic data, the structure of **5a** was determined as pregna-5,14-diene-3β,20-diol (Scheme 1), which was reported in our previous studies on calogenin glycosides (Pawar et al., 2007a). It was evident that **5a** was a product of dehydration at C-14 under the hydrolytic acidic conditions. Therefore, the aglycone in compound **5** was determined to be calogenin [(20*S*)-pregn-5-en-3β,14β,20-triol].



Scheme 2. Structures of compounds **1**–**7** (Cym: D-cymarose, The: D-thevetose, Ole: D-oleandrose, Dig: D-digitoxose, Glc: D-glucose).

Analysis of the acid hydrolysate of **5** confirmed the sugar moieties as D-oleandrose, D-thevetose, and D-glucose. Compound **5a** showed upfield chemical shifts at C-3 and C-20, as compared to **5**. Based on these glycosylation shifts, compound **5** was considered



Scheme 3. Structures of compounds **8–10** (The: D-thevetose, Ole: D-oleandrose, Glc: D-glucose).

Table 1
^{1}H and ^{13}C NMR spectroscopic data for aglycone parts of **1**, **2**, and **5–9** in pyridine-d^5 (values in parentheses denote *J* values in Hz).

	1		**2**[a]		**5–7**		**8**		**9**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	1.88, 2.32	37.3	1.02, 1.78	36.9	1.13, 1.78	37.9	1.86, 2.02	27.2	1.24, 1.83	36.7
2	1.63, 2.06	30.5	1.16, 1.88	29.7	1.79, 2.10	30.3	1.39, 1.89	29.9	1.28, 2.16	30.1
3	3.78 m	77.5	3.30 m	78.0	3.80 m	77.9	4.12 m	74.6	4.25 m	78.3
4	2.31, 2.49	39.4	2.11, 2.28	38.6	2.33, 2.69	39.6	1.37, 1.60	43.3	1.47, 1.83	33.2
5		139.7		138.9		139.9		84.9		176.1
6	5.38 br s	122.6	5.35 br s	122.1	5.54 br s	123.2	10.12 s	205.3	10.06 s	191.4
7	2.53, 2.56	28.1	1.11, 1.52	28.9	1.96, 2.67	28.2	2.70 m	63.5		138.1
8	1.94	37.1	2.11	35.6	1.81	37.7	3.02	46.9	2.83 d (12.4)	53.1
9	1.36	43.7	1.16	43.4	1.26 m	46.8	1.73	47.0	1.81	55.5
10		37.7		37.3		37.7		46.1		47.0
11	1.58, 1.94	26.9	1.29, 2.28	27.4	1.29, 1.74	20.1	1.82, 2.04	21.1	1.86, 2.53	22.1
12	4.93 dd (4, 12)	77.1	3.38	73.2	1.27, 1.43	41.1	2.11, 2.31	42.1	1.45	43.8
13		54.5		55.1		47.8		49.7		48.9
14		85.9		85.5		84.3		83.9		83.1
15	1.91	34.6	1.78	34.5	1.72	33.7	1.71, 2.22	32.7	2.25, 3.82	31.9
16	1.92, 1.98	24.5	1.88	24.4	1.77, 1.99	21.8	1.37, 1.52	23.5	1.28	21.9
17	3.21 m	58..6	3.55	56.8	1.62 m	57.6	1.68	57.1	1.63	57.5
18	0.92 s	11. 0	0.86 s	8.4	1.38 s	15.6	1.43 s	16.4	1.32 s	17.1
19	1.30 s	19.7	0.93 s	19.4	0.97 s	19.9	1.19 s	19.5	0.84 s	15.2
20		217.1		218.5	4.11 m	79.3	4.12	78.0	4.06	78.1
21	2.25 s	32.3	2.20 s	33.1	1.72 d (5.2)	22.1	1.43 d (8)	22.1	1.48 d (6.0)	23.7
12-O-tigloyl										
1*		167.9								
2*		129.6								
3*	7.15 d (6)	138.2								
4*	1.74 d (6)	14.7								
5*	1.98 s	12.7								

* Atoms of tigloyl substituent at C-12.
[a] Data recorded in $CDCl_3$.

to be a bisdesmoside. The anomeric proton of oleandrosyl unit (H-1′, δ_H 4.82) showed an HMBC correlation with C-3 (δ_C 77.9), while the anomeric proton of thevetose (H-1″, δ_H 4.87) showed a three-bond correlation with C-4′ (δ_C 83.7). This established the sugar se-

Table 2
^{1}H, and ^{13}C NMR spectroscopic data for sugar portions of **1** in pyridine-d^5, and **2** in $CDCl_3$ (values in parentheses denote *J* values in Hz).

	1		**2**	
	δ_H	δ_C	δ_H	δ_C
	Cym		Cym	
1′	5.26 br d (9.2)	96.6	4.80 br d (8.8)	95.9
2′	1.01, 1.66	37.5	1.56, 2.11	35.4
3′	4.07	78.4	3.75	77.1
4′	3.47	83.7	3.20	82.5
5′	4.22	69.3	3.83	68.5
6′	1.37 d (5.6)	18.8	1.25 d (6.4)	18.4
OMe	3.62 s	59.2	3.37 s	57.9
	Cym		Cym	
1″	5.12 br d (9.6)	100.7	4.71 br d (8.8)	99.6
2″	1.91, 2.60	37.6	1.66, 2.11	35.1
3″	4.07	78.3	3.78	77.1
4″	3.60	83.5	3.71	82.3
5″	4.22	69.6	3.87	68.4
6″	1.58 d (6)	19.0	1.23 d (6.4)	18.2
OMe	3.62 s	59.2	3.38 s	58.0
	The		The	
1‴	4.72 d (7.2)	106.3	4.25 d (7.6)	104.3
2‴	3.88	75.0	3.43	74.4
3‴	3.67	85.8	3.06	85.9
4‴	3.54	82.7	3.43	74.7
5‴	3.68	71.8	3.41	71.7
6‴	1.59 d (6)	18.9	1.20 d (6.4)	17.9
OMe	3.88 s	60.7	3.59 s	60.8
	Ole			
1⁗	4.94 d (11.2)	100.8		
2⁗	1.91, 2.60	37.7		
3⁗	3.49	81.9		
4⁗	3.51	76.6		
5⁗	3.62	73.3		
6⁗	1.58 d (6)	18.9		
OMe	3.49 s	57.4		

quence at C-3 to be 3-*O*-β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranoside. Further, HMBC correlations between the anomeric proton of the glucosyl unit (H-1‴, δ_H 4.94) and C-20 (δ_C 79.3) confirmed attachment of glucose at C-20. Based on these observations, the structure of compound **5** was established as the new bisdesmoside, calogenin-20-*O*-β-D-glucopyranosyl-3-*O*-β-D-thevetopyranosyl-(1→4)-β-D-oleandropyranoside. Compound **5** was named hoodigoside Y.

Compound **6** showed an $[M+Na]^+$ ion at *m*/*z* 905.4919 that was consistent with molecular formula $C_{46}H_{74}O_{16}$. The ^{1}H and ^{13}C NMR spectra of **6** were generally similar to those of **5**, except for the characteristic signals of a tigloyl moiety, a quartet at δ_H 7.04 (*J* = 7 Hz) for an olefinic proton, and two methyl signals at δ_H 1.69 (d, *J* = 6.4 Hz), and 1.86 (s). Further, the ^{1}H and ^{13}C NMR signals of **6** were consistent with those of hoodigoside V (Pawar et al., 2007a). Thus, the structure of compound **6** was determined as hoodigoside V [calogenin-20-*O*-β-D-glucopyranosyl-3-*O*-β-(4-*O*-tigloyl)-D-thevetopyranosyl-(1→4)-β-D-oleandropyranoside]. In our previous study, hoodigoside V was reported as a product of enzymatic hydrolysis. This is the first report of the natural occurance of hoodigoside V.

The HRESIMS data of compound **7** gave an $[M+Na]^+$ ion at *m*/*z* 1049.5655, which was 144 amu greater than that for hoodigoside V (**6**). This suggested the presence of an additional 3-methoxy-2,6-dideoxy sugar unit. From the ^{13}C NMR spectroscopic data, the aglycone moiety for compound **7** was identified as calogenin. Carbon chemical shifts for the tigloyl moiety were also present. HMBC correlations provided information about sugar linkages, in which the anomeric proton of the glucosyl unit (H-1⁗, δ_H 4.92) showed a correlation with C-20 (δ_C 79.3), confirming glycosylation at C-20. Further correlations between H-1′ (δ_H 5.31) and C-3 (δ_C 77.8), H-1″ (δ_H 5.13) and C-4′ (δ_C 84.9), H-1‴ (δ_H 4.82) and C-4′ (δ_C 84.0), and a three bond correlation between H-4‴ (δ_H 5.15/δ_C 75.9) of thevetose with C-1* of the tigloyl moiety (δ_C 167.5) established the sugar linkage at C-3 as 3-*O*-β-(4-*O*-tigloyl)-D-thevetopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside. Hence, the structure of hoodigoside Z (**7**) was deduced as calogenin-20-*O*-β-D-glucopyranosyl-3-*O*-β-(4-*O*-tigloyl)-D-thevetopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranoside.

In HRESIMS, compound **8** gave an $[M+Na]^+$ ion at *m*/*z* 937.4822, indicating a molecular formula of $C_{46}H_{74}O_{18}$. The FT-IR spectrum of **8** showed absorption bands at 3396, 1710, 1645, and 1071 cm^{-1} indicating the presence of a hydroxy, an aldehyde, an olefin, and an ester functionality. Upon a brief overview, the ^{1}H and ^{13}C NMR spectroscopic data of **8** were similar to those of **6**, suggestive of a steroidal bisdesmoside. However, an aldehyde proton at δ_H 10.12, which corresponded to a carbonyl carbon at δ_C 205.3 instead of the olefinic bond, and an additional oxygenated quaternary carbon (δ_C 84.9) were observed. This indicated a marked difference in the nature of functional groups of these two compounds. Further, analyses of the NMR spectra indicated the presence of nine methyls, nine methylenes, 21 methines, and seven quaternary carbons. Starting from the HMBC correlations of the angular methyl groups (C-18 and C-19), the ring systems of the aglycone moiety of **8** were established (Fig. 1). The H_3-18 (δ_H 1.43 s) showed an HMBC correlation with C-12 (δ_C 42.1), C-14 (δ_C 83.9), and C-17 (δ_C 57.1), which were consistent with those observed in compound **6**. However, the correlations between H_3-19 (δ_H 1.19 s) and C-5 (δ_C 84.9), C-10 (δ_C 46.1), C-9 (δ_C 47.0) and C-1 (δ_C 27.2) indicated presence of an

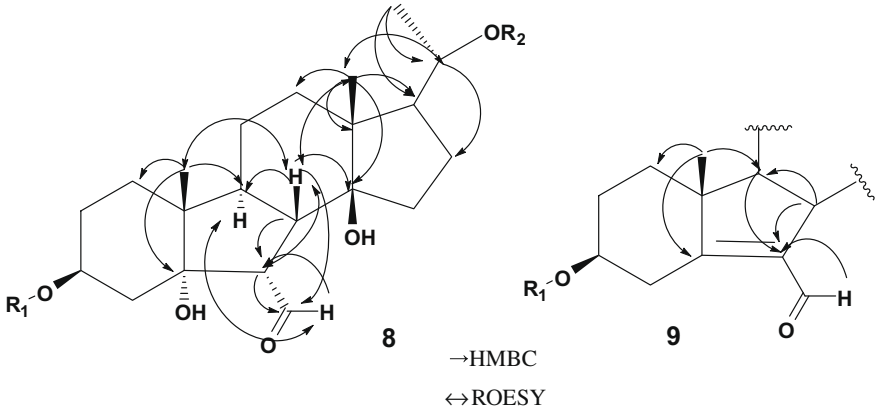


Fig. 1. HMBC and ROESY correlations for compound **8** and key correlations for **9**.

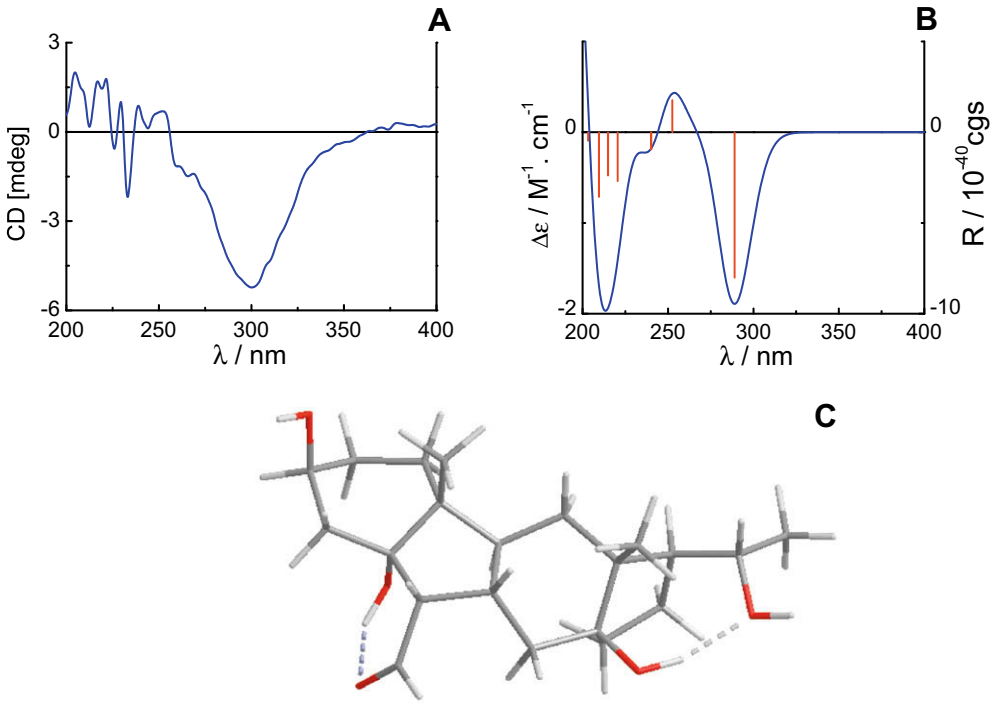


Fig. 2. (A) Experimental ECD spectrum for **8**; (B) calculated ECD spectrum for **8a**; and (C) optimized geometry of compound **8a**.

Table 3
^{1}H, and ^{13}C NMR spectroscopic data for sugar portions of compounds **5–9** in pyridine-d^5 (values in parentheses denote J values in Hz).

	5		**6**		**7**		**8**		**9**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
	Ole		Ole		Cym		Ole		Ole	
1′	4.82 br d (9.2)	98.3	4.82 br d (9)	98.3	5.31 br d (9.6)	97.2	4.71 br d (8.4)	99.3	4.91 br d (6.4)	98.6
2′	1.77, 1.87	38.1	1.80, 1.83	38.2	1.76, 2.33	37.5	1.74, 2.34	37.7	1.86,1.89	38.0
3′	3.62	79.8	3.61	79.8		78.3	3.57	79.7	3.61	79.8
4′	3.65	83.7	3.67	84.3	3.48	83.7	3.61	84.0	3.61	84.2
5′	3.65	72.2	4.18	72.2	4.22	69.3	4.14	72.2	4.25	72.2
6′	1.79 d (5.8)	19.3	1.75 d (6)	19.3	1.37 d (6.4)	18.8	1.70 d (6.8)	19.2	1.74 d (5.6)	19.2
OMe	3.52 s	57.5	3.54 s	57.7	3.56 s	59.1	3.45 s	57.6	3.55 s	57.8
	The		The		Cym		The		The	
1″	4.87 d (7.6)	104.4	4.96 d (8)	104.5	5.26 br d (7.6)	101.3	4.92 d (8.0)	104.4	4.98 d (7.6)	104.5
2″	3.87	75.4	3.98	75.2	1.76, 2.36	37.3	3.90	75.2	4.04	76.1
3″	3.59	88.4	3.71	85.4		78.3	3.65	85.3	3.65	85.3
4″	3.57	76.3	5.25 t (10)	76.0	3.56	83.5	5.19	76.0	5.15	76.0
5″	3.69	73.1	3.74	70.7	4.22	69.5	3.69	70.7	3.70	70.7
6″	1.43 d (5.6)	18.8	1.36 d (6)	18.4	1.38 d (5.6)	18.9	1.34 d (5.6)	18.3	1.36 d (6.0)	18.4
OMe	3.87 s	61.3	3.69 s	60.7	3.62 s	59.2	3.67 s	60.6	3.68 s	60.7
					The					
1‴					4.92 d (8)	106.3				
2‴					4.22	75.3				
3‴					3.68 m	84.8				
4‴					5.22 t (9)	75.3				
5‴					3.80	70.6				
6‴					1.35 d (6.4)	18.4				
OMe					3.70 s	60.7				
			Tigl		Tigl		Tigl		Tigl	
1*			–	167.5	–	167.5	–	167.5	–	167.5
2*			–	129.2	–	129.1	–	129.2	–	129.2
3*			7.05 d (6.8)	138.4	7.03 d	138.4	7.04 d (7.2)	138.3	7.06 d (6.8)	138.4
4*			1.68 d (7.6)	14.6	1.68 d (6)	14.7	1.69 d (7.0)	14.6	1.70 d (6.4)	14.6
5*			1.91 s	12.6	1.86 s	12.7	1.89 s	12.6	1.90 s	12.6
	Glc		Glc		Glc		Glc		Glc	
1‴′	4.94 d (7.6)	105.2	4.91 d (7)	105.3	4.91 d (6.8)	105.0	4.86 d (6.8)	105.0	4.91 d (6.4)	106.2
2‴′	3.87	75.5	3.94	75.5	3.90	75.2	3.90	75.1	3.95	75.3
3‴′	3.87	78.8	3.94	78.9	4.08	79.9	4.08	79.6	3.94	79.0
4‴′	4.13	71.7	3.74	71.8	4.14	72.3	4.14	71.8	4.25	72.0
5‴′	4.13	78.3	4.15	78.3	3.87	78.9	3.87	78.8	3.94	78.7
6‴′	4.31 m, 4.49 d (11.2)	63.1	4.34 m, 4.53 br d (11.0)	63.1	4.32 dd (4.8, 11.6), 4.50 d (10.0)	63.7	4.32 dd (4.8, 11.6), 4.50 d (10.0)	63.1	4.40 m, 4.57 d (10.8)	63.3

* Atoms of tigloyl substituent on the terminal sugar moiety.

oxygenated quaternary carbon at C-5. The aldehydic proton H-6 (δ_H 10.12) showed a two-bond correlation to C-7 (δ_C 63.5) that indicated attachment of the aldehyde functionality at C-7. Based on these observations, a preliminary structure for the aglycone was contemplated as 3,5,14,20-tetrahydroxy-5(6→7)*abeo*-pregnan-7-al. Previously, orostanal, a related B ring *abeo* derivative of cholesterol, was reported from a marine sponge *Stelletta hivasaensis*, and the chemical shift values for rings A and B for compound **8** were comparable to those for orostanal (Miyamoto et al., 2001). The relative configuration of the aglycone was determined by the ROESY spectrum, in which correlations between H-7 (δ_H 2.70) and methines H-8 (δ_H 3.02), and H-4β (δ_H 1.37) confirmed the β-orientation of H-7. Further, interactions between methyl protons H_3-19 (δ_H 1.19)/H-8 (δ_H 3.02), and H-6 (δ_H 10.12)/H-9 (δ_H 1.73), in turn indicated the α-orientation of the aldehyde moiety. A ROESY correlation was also observed between H-20 (δ_H 4.12) and H_3-18 (δ_H 1.43) that confirmed the β-orientation of H-20, and thus indicated the configuration at C-20 to be 20*S*.

Further the absolute configuration of the aglycone (**8a**) was determined by comparison of its theoretically calculated electronic circular dichroism (ECD) spectrum, and the experimental ECD of the glycoside (**8**), assuming both the aglycone and the glycoside exhibit similar ECD spectra. This assumption was based on the fact that the sugar moieties in glycosides (e.g., flavanone or 3-hydroxyflavanone glycosides) (Gaffield, 1970) generally have little effect on their overall experimental ECD spectra. The calculated results showed that the negative Cotton effect (CE) at 293 nm attributable to the aldehyde $\pi\rightarrow\pi^*$ transition in **8a**, corresponds to the negative CE at 300 nm in the experimental ECD spectrum of **8** in methanol. Therefore, the aglycone possesses 5*S*, 7*S*, 8*S*, 20*S* absolute configuration (Fig. 2). The major reason using the aglycone for theoretical ECD calculation was to reduce the 'size' of the molecule and hence conformational "freedom" in order to facilitate practical calculations with available computational methods. In addition, the aglycone of **8** is not readily accessible by chemical degradation due to its acid-lability.

The anomeric region of the ^{1}H NMR spectrum of **8** showed three doublets at δ_H 4.71 (J = 10.2 Hz), 4.92 (J = 8.0 Hz), and 4.88 (J = 7.6 Hz) corresponding to carbons at δ_C 99.3, 104.4 and 105.0, respectively. Based on the 2D NMR spectroscopic data, and the hydrolysis studies, the sugar moiety with the anomeric proton at δ_H 4.71 was recognized as D-oleandrose, the one with the anomeric proton at δ_H 4.92 as D-thevetose, and the third sugar as D-glucose. The chemical shift values for the sugar protons and carbons were consistent with those of compound **6** (see Table 3). HMBC correlations facilitated determination of the sugar linkages. The HMBC correlations of H-1‴ of glucose (δ_H 4.88) to C-20 (δ_C 78.0) confirmed attachment of glucose at C-20. Further, the correlations of H-1′ of oleandrose (δ_H 4.71) with C-3 (δ_C 74.6), H-1″ of thevetose (δ_H 4.92) with C-4′ (δ_C 84.0) and H-4″ of thevetose (δ_C 75.9) with δ_C 167.5 indicated the presence of the sugar chain at C-3 as β-(4-*O*-tigloyl)-D-thevetopyranosyl-(1→4)-β-D-oleandropyranoside. Thus, the structure of **8** was elucidated as a novel *abeo*-sterol glycoside, 5α,14β-dihydroxy-5(6→7)*abeo*-pregnan-7α-al-20-*O*-β-D-glucopyranosyl-3-*O*-β-(4-*O*-tigloyl)-D-thevetopyranosyl-(1→4)-β-D-oleandropyranoside, and was named hoodistanaloside A.

The HRESIMS for compound **9** showed a sodiated molecular ion at *m*/*z* 919.4671, which was 18 amu less than that of **8**, indicating loss of a water molecule. The molecular formula of $C_{46}H_{73}O_{17}$ was further corroborated by the ^{13}C NMR spectrum that indicated the presence of an additional pair of olefinic carbon atoms at δ_C 176.1 and 138.1. Compared to compound **8**, the aldehydic carbon was shifted upfield at δ_C 191.4 (Table 1) in compound **9**. This, along with the other important HMBC correlations (Fig. 1), defined the double bond at Δ^{5-7}, and the aldehyde functionality at C-7. The chemical shift values for other atoms of the aglycone part (**9a**), and sugar moieties of compound **9** were comparable to those of compound **8**. Hence, compound **9** was considered as a C-5 dehydrated product of **8**. Based on this, the structure of hoodistanaloside B (**9**), a new natural product, was deduced as 14β-hydroxy-5(6→7)-*abeo*-pregn-5-en-7-al-20-*O*-β-D-glucopyranosyl-3-*O*-β-(4-*O*-tigloyl)-D-thevetopyranosyl-(1→4)-β-D-oleandropyranoside.

Compound **10** showed an $[M+H_2O]^+$ ion in HRESIMS at 594.4703 corresponding to a molecular formula of $C_{35}H_{60}O_6$. Comparison of NMR spectroscopic data with reported information established the structure of compound **10** as stigmast-5-en-3-*O*-β-D-glucopyranoside (Kadowaki, 2003).

3. Concluding remarks

Previous phytochemical studies have shown that *H. gordonii* contains an abundance of pregnane glycosides, which comprise of hoodigogenin A, and calogenin as the aglycones (Dall'Acqua and Innocenti, 2007; Pawar et al., 2007a, 2007b). Hoodigogenin A is an unique pregnane derivative from *Hoodia*, due to the tigloyl ester substitution at C-12. Although calogenin bisdesmosides have been reported from other plants in Asclepiadaceae family (Al-Yahya et al., 2000; Deepak et al., 1996; Siciliano et al., 2005; Sigler et al., 2000; Srivastava et al., 2007; Trivedi et al., 1989), the tigloyl functionality attached at the C-4 of terminal sugar is a distinctive feature of calogenin glycosides from *H. gordonii*. The present communication reports structures of two new hoodigogenin A glycosides (**1**, **2**), two new glycosides of calogenin (**5**, **7**) and two glycosides (**8**, and **9**), which comprise of novel chemotypes of aglycones, namely hoodistanal (**8a**) and dehydrohoodistanal (**9a**). Natural occurrences of such 6-5-6-5 fused ring sterols, like compounds **8** and **9** are rare. There have been only two reports of *abeo*-sterols from marine sponges (Miyamoto et al., 2001; Wei et al., 2007), and one from a terrestrial plant (Lin et al., 1998). Hoodistanalosides A and B are the first two naturally occurring glycosides, comprising a 5(6→7)*abeo*-sterol aglycone.

4. Experimental

4.1. General experimental procedures

NMR spectra were recorded on a Varian AS400 NMR spectroscope at 400 MHz (^{1}H) and 100 MHz (^{13}C). Proton and carbon chemical shift values are relative to internal standard TMS and were acquired in C_5D_5N and $CDCl_3$. High-resolution mass spectra (positive) were acquired using electro-spray ionization (ESI) source, on an Agilent Series 1100 SL spectrometer. Optical rotations were measured on the Autopol IV polarimeter and specific rotations are expressed as deg.10.g^{-1}.cm^2. The ECD spectrum of compound **8** was recorded on Jasco J-715 CD spectropolarimeter, in MeOH at 0.5, 1 and 2 mg/mL. TLC analyses were carried out on silica gel 60 F_{254} plates (Merck, Germany) using $CHCl_3$/MeOH/H_2O (90:10:0.5, 90:12:1) and C-18 reversed phase silica TLC plates (Analtech, USA) with MeOH/H_2O (70:30, 75:25). Compounds were visualized by spraying with anisaldehde-H_2SO_4 followed by heating at 105 °C for 1–2 min Column chromatography (CC) was carried out on silica gel (JT Baker, 40–60 μm for flash chromatography) and reversed phase C18 silica gel (Polarbond, JT Baker). Sugars were identified by GC–MS of their alditol acetates. D-thevetose and D-glucose were obtained from Sigma–Aldrich (USA).

4.2. Plant material

Powdered whole aerial parts of *H. gordonii* were purchased from Psychoactive Herbs Ltd. (www.psychoactiveherbs.com) in September 2005. The plant material was authenticated by compar-

ing with an authentic sample of *H. gordonii* provided by the Missouri Botanical Garden, Missouri, USA. Chemical authentication was done by comparing the HPLC fingerprint of the methanol extract of the purchased material with that of the authentic sample of *H. gordonii*. A voucher specimen (voucher no. 2799) has been deposited in the repository of National Center for Natural Product Research.

4.3. Extraction and isolation

Coarsely powdered *H. gordonii* material (4.75 kg) was extracted by percolation with $CHCl_3$ (4 × 4 L). The extracts were combined and concentrated to obtain a thick mass (402.1 g). The latter was dissolved in MeOH/H_2O (95:5) and partitioned with hexanes. The polar fraction (136 gm) was subjected to VLC on silica gel (1500 gm) by eluting with gradients of $CHCl_3$/MeOH/H_2O from 100:4:0.5 (3 L), up to 90:12:0.5 (by increasing MeOH and reducing $CHCl_3$, each by 1% increments), to generate eight sub-fractions (fr.1–fr.8).

Upon storing overnight at room temperature, a white precipitate was formed in sub-fraction 8. This precipitate was washed with hexanes and acetone to obtain compound **10** (71 mg). Upon chromatographic separation on an RP-18 column with MeOH/H_2O (7:3), as eluant sub-fraction 2 afforded compound **2** (52 mg). Sub-fraction 4 was subjected to multiple reversed phase column chromatography separations, using MeOH/H_2O (7:3) to obtain compounds **1** (75 mg), **3** (8 mg), and **4** (206 mg). Sub-fraction 6 was subjected to silica gel CC with linear gradients of $CHCl_3$/MeOH (99:1) to isolate the major compound **6** (1.3 gm), $CHCl_3$/MeOH (97:3) to obtain compound **8** (38 mg), and $CHCl_3$/MeOH (95:5) to yield compound **9** (5.5 mg). Sub-fraction 8 was subjected to reversed phase (C-18) CC using MeOH/H_2O (6:4), followed by repeated purification on silica gel column using an isocratic solvent system of $CHCl_3$/MeOH/H_2O (90:8:0.5) that yielded compounds **5** (41 mg) and **7** (26 mg).

4.3.1. Hoodigoside W (**1**)

White amorphous powder; $[\alpha]_D^{25}$ +3.3 (*c* 0.3, MeOH); IR (NaCl) ν_{max} 3392, 2933, 1713, 1648, 1067 cm^{-1}; for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m*/*z* 1045.5711 (calcd for $C_{54}H_{86}O_{18}Na$, $[M+Na]^+$ *m*/*z* 1045.5706).

4.3.2. Hoodigoside X (**2**)

White amorphous powder; $[\alpha]_D^{25}$ −20.0 (*c* 0.3, MeOH); IR (NaCl) ν_{max} 3398, 2930, 1713, 1646, 1071 cm^{-1}; for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m*/*z* 819.4571 (calcd for $C_{42}H_{68}O_{14}Na$, $[M+Na]^+$ *m*/*z* 819.4501).

4.3.3. Hoodigoside Y (**5**)

White amorphous powder; $[\alpha]_D^{25}$ −16.7 (*c* 0.25, MeOH); IR (NaCl) ν_{max} 3395, 2932, 1711, 1646, 1076 cm^{-1}; for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 3; HRESIMS *m*/*z* 823.4519 (calcd for $C_{41}H_{68}O_{15}Na$, $[M+Na]^+$ *m*/*z* 823.4450).

4.3.4. Hoodigoside V (**6**)

White amorphous powder; $[\alpha]_D^{25}$ −12.0 (*c* 0.23, MeOH); IR (NaCl) ν_{max} 3393, 2932, 1711, 1650, 1060 cm^{-1}; for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 3; HRESIMS *m*/*z* 905.4882 (calcd for $C_{58}H_{94}O_{25}Na$, $[M+Na]^+$ *m*/*z* 905.4869).

4.3.5. Hoodigoside Z (**7**)

White amorphous powder; $[\alpha]_D^{25}$ −22.4 (*c* 0.25, MeOH); IR (NaCl) ν_{max} 3389, 2934, 1710, 1068 cm^{-1}; for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 3; HRESIMS *m*/*z* 1049.5655 (calcd for $C_{53}H_{86}O_{19}Na$, $[M+Na]^+$ *m*/*z* 1049.5583).

4.3.6. Hoodistanaloside A (**8**)

White amorphous powder; $[\alpha]_D^{25}$ −22.7 (*c* 0.3, MeOH); IR (NaCl) ν_{max} 3368, 2932, 1710, 1690, 1059 cm^{-1}; for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 3; HRESIMS *m*/*z* 937.4822 (calcd for $C_{46}H_{74}O_{18}Na$, $[M+Na]^+$ *m*/*z* 937.4815).

4.3.7. Hoodistanaloside B (**9**)

White amorphous powder; $[\alpha]_D^{25}$ −27.3 (*c* 0.2, MeOH); IR (NaCl) ν_{max} 3445, 2940, 1714, 1687, 1057 cm^{-1}; for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 3; HRESIMS *m*/*z* 919.4663 (calcd for $C_{46}H_{72}O_{17}Na$, $[M+Na]^+$ *m*/*z* 919.4661).

4.4. Acid hydrolysis of glycosides

A mixture of compounds **1**, **3**, and **4** (50 mg) was hydrolyzed with 0.05 N HCl in 50% 1,4-dioxane at 90 °C for 2 h. The resulting mixture was partitioned with $CHCl_3$. The $CHCl_3$ layer was dried and subjected to CC on silica gel by eluting with gradients of $CHCl_3$ and MeOH to obtain aglycone **1a** (13 mg). Similarly, compound **2** (10 mg) and compound **5** (10 mg) were hydrolyzed using the same protocol to obtain aglycones **2a** (1 mg) and **5a** (1.8 mg), respectively (Scheme 1). **2a** and **5a** were purified from the reation mixture by preparative TLC using $CHCl_3$:MeOH (95:5). Structures of aglycones **1a**, **2a**, and **5a** were established by NMR spectroscopy.

4.5. Sugar analysis

Each compound (ca. 1 mg) was hydrolyzed as described above, and the polar fraction obtained after partitioning with $CHCl_3$ was used for sugar analyses. Alditol acetates of sugars were prepared (Sawardekar et al., 1965). Thus, the sugar portion was dissolved in water and reduced with $NaBH_4$ at room temperature for 3 h, and the reaction mixture was neutralized with AcOH. To the dried material, equal amounts of pyridine and Ac_2O were added and heated at 95 °C for 1 h. After cooling, H_2O was added and alditol acetates were extracted with $CHCl_3$, which were subjected to GC–MS analysis (column, JW DB-5, 30 m × 0.25 mm, 0.25 μm; carrier gas He; injection temp. 280 °C, detection temperature 280 °C, column temperature; 150 °C (1 min), 10 °C/min to 250 °C, retention times: t_R cymaritol acetate 5.34 min, t_R oleandritol acetate 5.65 min, t_R thevititol acetate 7.63 min, t_R glucitol acetate 10.48 min). Glucose (1.7 mg), thevetose (2.3 mg), cymarose (5.6 mg) and oleandrose (2.7 mg) were obtained from hydrolysis of subfraction 4 (300 mg) by using the method described in Section 4.4. The absolute configurations of thevetose and glucose were determined via GC–MS as described before (Hara et al., 1987; Pawar et al., 2007b). Retention times of acetylated thiazolidine derivatives of glucose (t_R 23.44 min) and thevetose (t_R 16.29 min) were compared with those of the acetylated thiazolidine derivatives synthesized from standard sugars (t_R D-glucose 23.4 min, t_R L-glucose 25.38 min, t_R D-thevetose 16.33 min). Both cymarose and oleandrose were considered to be in D-form based on their specific rotations (D-cymarose $[\alpha]_D^{25}$ +55.55 (*c* 0.9, H_2O); and D-oleandrose $[\alpha]_D^{25}$ −15.90 (*c* 0.7, H_2O)).

4.6. Electronic circular dichroism calculations

For theoretical ECD calculations for compound **8a**, ground state geometries have been optimized at B3LYP/6-31G* level in gas phase, vibrational harmonic frequencies have been calculated to verify the minimum time dependent density functional theory (TDDFT), and have been performed at B3LYP-SCRF/6-31G*//B3LYP/6-31G* level with "COnductor-like continuum Solvent MOd-

el" (COSMO) (Ding et al., 2007; Klamt and Schurmann, 1993; Pecul et al., 2005; Sinnecker et al., 2006). All computations were carried out at 298 K by using the Gaussian03 program package. The calculated rotatory strengths R in dipole length form (R_{len}) were simulated into an ECD curve by using the Gaussian function:

$$\Delta \in (E) = \frac{1}{2.297 \times 10^{-39}} \frac{1}{\sqrt{2\pi\sigma}} \sum_{i}^{A} \Delta E_i R_i e^{[-(E-\Delta E_i/2\sigma)]^2}$$

where σ is the width of the band at $1/e$ height and ΔE_i and R_i are the excitation energies and rotatory strengths for transition i, respectively $\sigma = 0.10$ eV was used.

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