An appetite suppressant from *Hoodia* species

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Abstract

Two new pregnane glycosides were isolated by fractionation of the dried stems of *Hoodia gordonii*. Their structures were determined as 3β-[β-D-thevetopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranosyloxy]-12β-tigloyloxy-14β-hydroxypregn-5-en-20-one (**1**) and 3β-[β-D-cymaropyranosyl-(1→4)-β-D-6-deoxy-3-*O*-methylallopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranosyloxy]-12β-tigloyloxy-14β-hydroxypregn-5-en-20-one (**2**) on the basis of spectroscopic studies and conversion to known compounds. Compounds **1** and **2** were also isolated from *Hoodia pilifera*. Compound **1** was tested for its appetite-suppressant properties in rats by oral gavage at 6.25 to 50 mg/kg and the results showed that all doses resulted in a decrease of food consumption over an eight day period and a body mass decrease when compared to the control sample receiving only the vehicle. In a comparative study against a fenfluramine control sample, compound **1** resulted in a reduction in food intake over the study period, with a concomitant overall decrease in body weight while fenfluramine resulted in a small decrease in food intake, but an *increase* in body weight (though less than control group) over the same period of time.

Keywords: Hoodia pilifera, Hoodia gordonii, Apocynaceae, ghaap, appetite suppressant, pregnane glycoside, cymarose, thevetose, methyl β-lilacinobioside, 12-*O*-tigloyldigipurpurogenin II

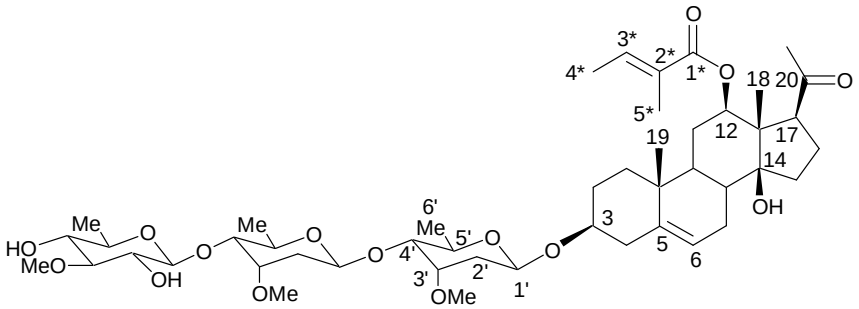
Introduction

Hoodia pilifera (L.f.) Plowes (Apocynaceae) is a succulent occurring in arid areas of southern Africa and is referred to by the indigenous people as ghaap, guaap, or ngaap. It was recorded that the plant [previously classified as *Trichocaulon piliferum* (Linné *fil*) N. E. Brown] had an insipid, yet cool and watery taste, and was eaten by the natives for the purpose of quenching their thirst (Pappe, 1862). The plant has been reported to be edible in its raw state or preserved in sugar (White, 1937) and has been described as the 'real ghaap' of the natives, who used it as a substitute for food and water (Marloth, 1932). The larger, hard-spined species *Hoodia gordonii* (Masson) Sweet ex Decne., is reportedly more rarely eaten and its lower status as a food is indicated by the disparaging names of 'muishondghaap' or 'jakkalsghaap' indicating that the plant is only fit for animals (White, 1937). The plant has a more bitter taste that is persistent and spreads around the mouth (Bruyns, 1993).

Studies conducted at the Council for Scientific and Industrial Research (CSIR, South Africa) identified extracts from *Hoodia* species, in particular *H. pilifera* and *H. gordonii*, as possessing appetite-suppressing properties. *Hoodia* preparations are currently highly publicised for their appetite-suppressant properties and several publications have appeared on the benefit sharing agreement between the CSIR and the South African San Council (WHO, 2006), appetite-suppressant properties of the plant extracts (van Heerden, 2002; Tulp, 2001), mode-of-action studies (MacLean, 2004) and analysis of the active ingredient in formulations (Avula, 2006). The present paper describes the original work done by the CSIR scientists on the isolation, structure elucidation and biological activity of two compounds extracted from *H. gordonii* and *H. pilifera*.

Results and discussion

Extraction of the dried milled aerial parts of *H. gordonii* with a methanol-dichloromethane solution, followed by solvent-solvent partitioning and extensive column chromatography, led to the isolation of compounds **1** and **2**.



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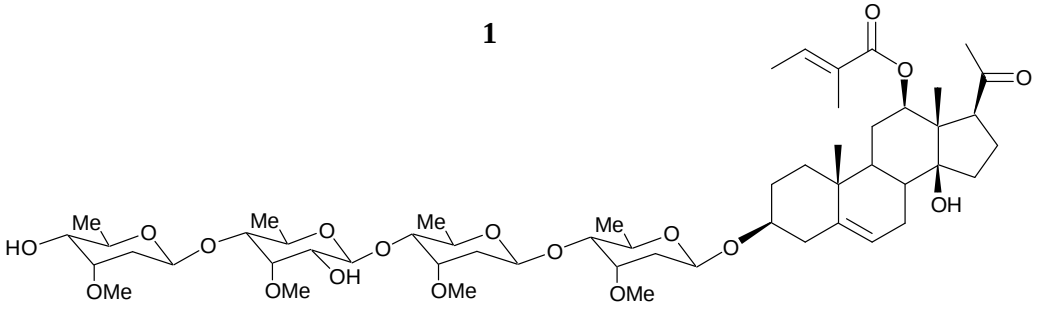


2

Glycoside **1** showed a molecular ion peak $[M+Na]^+$ at *m/z* 901.4943 in the positive HR-ESI-MS, in accordance with an empirical molecular formula of $C_{47}H_{74}O_{15}Na$ while compound **2** showed a molecular ion peak $[M+Na]^+$ at *m/z* 1045.5631, in accordance with an empirical molecular formula of $C_{54}H_{86}O_{18}Na$. These molecular formulae were supported by the ^{13}C NMR spectrum in which 47 resonances were observed for glycoside **1** with the signals consisting of eleven methyl, nine methylene, twenty methine and seven quaternary carbon signals and 54 resonances observed for glycoside **2** consisting of thirteen methyl, ten methylene, twenty-four methine and seven quaternary atoms.

Table 1: ^{1}H NMR data (300.13 MHz, $CDCl_3$) of glycosides **1** and **2**

		Glycoside 1	Glycoside 2
	Position	δ_H (mult., *J*)	δ_H (mult., *J*)
Aglycone moiety			
	3	3.52 (*m*)	3.50 (*m*)
	6	5.38 (*m*)	5.34 (*m*)
	12	4.61 (*dd*, *J* 11.5, 4.1)	4.59 (*dd*, 11.6, 4.3)
	17	3.16 (*dd*, 9.3, 9.3)	3.16 (*dd*, 9.3, 9.3)
	18	1.03 (s)	1.01 (*s*)
	19	0.95 (*s*)	1.93 (*s*)
	21	2.16 (*s*)	2.14 (*s*)
Tigloate moiety			
	3*	6.89 (*qq*, 7.1, 1.5)	6.87 (*qq*, 7.1, 1.5)
	4*	1.81 (*dq*, 7.1, 1.2)	1.78 (*dq*, 7.1, 1.2)
	5*	1.85 (*dq*, 1.6, 1.2)	1.83 (*dq*, 1.6, 1.2)
Carbohydrate moiety			
		β-cym	β-cym
	1'	4.82 (*dd*, 9.4, 2.1)	4.78 (*dd*, 9.6, 2.1)
	$2'_{eq}$	2.06 (*ddd*, 13.8, 3.7, 2.1)	2.05 (*ddd*, 13.8, 3.7, 2.1)
	$2'_{ax}$	1.55 (*ddd*, 13.8, 9.4, 2.6)	1.55 *ddd*, 13.8, 9.4, 2.6
	3'	3.78 (*ddd*, 3.7, 2.9, 2.6)	3.75 (*ddd*, 3.7, 2.9, 2.6)
	4'	3.18 (*dd*, 9.4, 2.9)	3.18 (*m*)
	5'	3.82 (*dd*, 9.4, 6.3)	3.81 (*dd*, 9.4, 6.3)

6'	1.28[a] (*d*, 6.3)		1.16[a] (*d*, 6.2)
3'-OMe	3.41[b] (*s*)		3.38[b] (*s*)
	β-cym		β-cym
1"	4.73 (*dd*, 9.4, 2.1)		4.70 (*dd*, 9.7, 2.1)
$2"_{eq}$	2.11 (*ddd*, 13.8, 3.7, 2.1)		2.10 (*ddd*, 13.8, 3.7, 2.1)
$2"_{ax}$	1.60 (*ddd*, 13.8, 9.4, 2.6)		1.59 (*ddd*, 13.8, 9.4, 2.6)
3"	3.76 (*ddd*, 3.7, 2.9, 2.6)		3.71 (*ddd*, 3.7, 2.9, 2.6)
4"	3.24 (*dd*, 9.4, 2.9)		3.18 (*m*)
5"	3.90 (dd, 9.4, 6.3)		3.87 (*dd*, 9.4, 6.3)
6"	1.24[a] (*d*, 6.3)		1.24[a] (*d*, 6.1)
3"-OMe	3.39[b] (*s*)		3.37[b] (*s*)
	β-the		β-mda
1'"	4.27 (*d*, 7.7)		4.23 (*d*, 7.8)
$2"_{eq}$	----		----
$2"_{ax}$	3.47 (*dd*, 8.0, 7.7)		3.41 (*dd*, 8.0, 3.0)
3'"	3.10 (*dd*, 8.0, 2.9)		3.18 (*m*)
4'"	3.18 (*dd*, 9.3, 2.9)		3.18 (*m*)
5'"	3.35 (*dd*, 9.3, 6.3)		3.32 (*dd*, 9.3, 6.3)
6'"	1.18[a] (*d*, 6.3)		1.24[a] (*d*, 6.2)
3'"-OMe	3.62 (*s*		3.56 (s)
			β-cym
1""		----	4.78 (*dd*, 9.7, 2.1)
$2""_{eq}$		----	2.08 (*ddd*, 13.8, 3.7, 2.1)

2''''$_{ax}$		1.57 *ddd*, 13.8, 9.7, 2.6)
3''''		3.57 (*ddd*, 3.7, 2.9, 2.6)
4''''	----	3.20 (*m*)
5''''	----	3.54 (*dd*, 9.4, 6.3)
6''''	----	1.22[a] *d*, 6.3)
3''''-OMe	----	3.37[b] (*s*)

[a, b] Assignments are interchangeable within each column

Table 2: ^{13}C NMR data (75.25 MHz, in $CDCl_3$) for the glycosides **1** and **2**

	Glycoside 1	Glycoside 2
Carbon number	δ_C	δ_C
Aglycone moiety		
1	37.0	37.1
2	29.4	29.4
3	77.2	77.0[a]
4	38.6	38.6
5	138.9	139.0
6	121.9	121.9
7	27.3	27.3
8	35.3	36.6
9	43.0	43.0
10	37.2	37.2
11	26.0	26.0
12	75.9	75.8
13	53.7	53.7
14	85.7	85.8
15	34.4	34.4
16	24.3	24.3
17	57.2	57.1
18	9.8	9.9
19	19.3	19.3

20	216.8	217.0
21	33.0	33.1
Tiglate moiety		
1*	167.6	167.6
2*	128.7	128.7
3*	137.7	137.7
4*	14.4	14.4
5*	12.1	12.1
Carbohydrate moiety		
	β-cym	β-cym
1'	95.8	95.9
2'	35.6	35.5
3'	77.0	77.3[a]
4'	82.6	82.7
5'	68.5	68.5
6'	18.1	18.1[b]
3'-OMe	57.9	57.9[c]
	β-cym	β-cym
1"	99.5	99.5
2"	35.2	35.4
3"	77.0	77.4[a]
4"	82.5	82.5
5"	68.3	68.3

6"	18.4	18.0[b]
3"-OMe	57.1	57.1[c]
	β-the	β-mda
1'''	104.3	104.1
2'''	74.6	74.0
3'''	85.3	84.0
4'''	74.6	81.7
5'''	71.6	71.0
6'''	17.7	18.2[b]
3'''-OMe	60.6	60.0
		β-cym
1''''	----	98.3
2''''	----	33.9
3''''	----	77.4[a]
4''''	----	72.5
5''''	----	71.2
6''''	----	18.0[b]
3''''-OMe	----	58.1[c]

[a, b, c] Assignments are interchangeable within each column

The ^{1}H and ^{13}C data (Tables 1 and 2) for compound **1** pointed to the presence in the molecule of two *O*-methylated 2,6-dideoxy sugars and a single *O*-methylated 6-deoxy sugar. The β-linkages of the sugars of the glycoside **1** were revealed by the magnitude of the ^{1}H-^{1}H coupling constants, J = 9.4, 9.4 and 7.7 Hz, of the anomeric

proton signals (atom numbers 1′,1″,1‴, respectively). The magnitude of the coupling constants of the anomeric protons between the α and β configurations have been well documented in literature (Steyn, 1989). Literature of steroidal glycosides indicate that most β-linked 2,6-dideoxy sugars have the D-configuration, whereas the α-linked sugars are mostly L-sugars (Vleggaar, 1993). The methyl groups of the 6-deoxy sugars were at δ_H 1.28, 1.24, 1.18 and methoxy groups of the sugars were at δ_H 3.41, 3.39, 3.62. The remainder of the signals in the ^{1}H NMR spectrum of the glycoside exhibited complex structure. In this instance, the proton-proton connectivity pattern and chemical shift values could be obtained from ^{1}H-^{1}H COSY experiments.

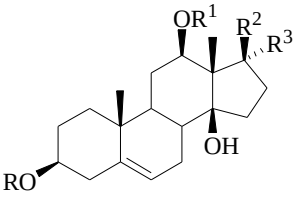
Sequence information of the sugars of the glycoside and the substitution pattern of the aglycone were deduced from HMBC experiments. The fact that the first and second carbohydrate units were 2-deoxy carbohydrates was proven by the correlations observed between the anomeric protons and the resonances for the C-2 carbon atom (C-1′ anomeric proton correlates to the resonance of C-2′, C-1″ anomeric proton correlates to the resonance of C-2″). The sequence of the carbohydrate moieties was established by the 3-bond correlations obtained across the glycosidic bonds. These correlations were between the C-1″ anomeric proton (δ_H 4.73) and the C-4′ resonance at δ_C 82.6 which confirmed that the first and second 2-deoxy cymarose units were linked to each other, correlation between the C-1‴ anomeric proton (δ_H 4.27) and the C-4″ resonance at δ_C 82.5 established that the 6-deoxy thevetose unit was attached to the second cymarose unit. The methoxy group at position C-3 of the carbohydrates was confirmed by the correlations observed between the methoxy protons and the resonances for C-3′, C-3″ and the C-3‴ carbon signals.

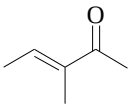
The presence of the tigloate ester was recognised from the analysis of ^{1}H and ^{1}H-^{1}H COSY NMR spectra. A coupling (J = 1.5 Hz) was observed between the vinylic proton which resonates at δ_H 6.89 and the protons of the methyl group located on a sp^2 carbon atom (δ_H 1.85).

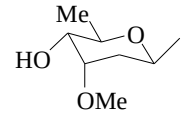
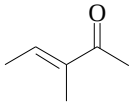
Assignments were also made for the aglycone moiety using the data from the ^{1}H-^{1}H COSY experiment. These were for H-3 which resonates at δ_H 3.52, H-17 at δ_H 3.16 and H-12 at δ_H 4.61. The broadness of the resonance for H-3 and the coupling constants of J = 11.5 and 4.1 Hz for the resonance corresponding to H-12 indicated that the functional groups at C-3 and C-12 both have a β stereochemistry. The three-proton singlets observed for the glycoside at δ_H 0.95, 1.03 and 2.16 were assigned to the methyl groups of the aglycone. The remaining signals in the ^{1}H NMR spectrum exhibited fine structure and were attributed to the aglycone unit. The following correlations observed in ^{1}H-detected heteronuclear multiple bond connectivity (HMBC) spectrum led to the confirmation of the following substitution pattern on the aglycone: (i) correlation between the C-12 proton (δ_H 4.61) and the singlet resonance (δ_C 167.6) of the C-1 of the tigloate ester, established that the ester is attached to the C-12 position of the steroid, (ii) the three bond correlation between the C-3 proton (δ_H 3.52) and the resonance (δ_C 95.8) of the C-1′ anomeric carbon confirmed the carbohydrate moiety is attached to the C-3 position of the steroid.

Mild acid hydrolysis of the trisaccharide glycoside **1** (0.05 M H_2SO_4-50% MeOH, 50 °C) afforded the hydrolysis products *viz.* the aglycone moiety **3**, the monoglycoside **4**, the monosaccharide **6a** and the disaccharide units **7a** and **7b**. The hydrolysis products

obtained were identified using NMR spectroscopy and the comparison of these data as well as the physical data to that of published data.



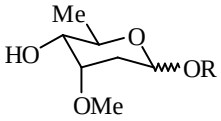
(3) R = H , R^1 =  , $R^2 = COCH_3$, R^3 = H

(4) R =  , R^1 = 

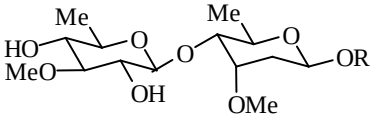
$R^2 = COCH_3$, R^3 = H

(5a) R = H, R^1 = H, $R^2 = COCH_3$, R^3 = H

(5b) R = H, R^1 = H, R^2 = H, $R^3 = COCH_3$



(6a) R = H
(6b) R = OMe



(7a) R = H
(7b) R = OMe

The aglycone moiety was identified as 12-*O*-tigloyldigipurpurogenin II **(3)** based on NMR analysis (Table 3). The C-12 tigloyl ester group of aglycone **3** was chemically removed using methanolic KOH, which also resulted in the epimerization at the C-17 position. The C-17 epimers, compounds **5a** and **5b,** were obtained as a mixture in a 1:3 ratio. This was determined using the integral ratio of the C-18 signals for compounds **5a** and **5b** in the ^{1}H NMR spectrum and has been previously described (Rubin, 1963). A similar epimeric ratio was also obtained by Mitsuhashi (1965) during the base hydrolysis of 12-*O*-benzoyldigipupurogenin II. The epimers were separated by silica flash chromatography and compared favourably with the published data for digipurpurogenin II (**5a**) (Tschesche, 1961).

Table 3: ^{1}H and ^{13}C NMR data ($CDCl_3$) for aglycone **3**

Carbon	^{13}C NMR chemical shift (δ_X)	^{1}H NMR chemical shift (δ_H)	^{1}H-^{13}C connectivity (HMBC)	^{1}H-^{1}H connectivity (COSY)
1	36.8	1.12, 1.80	C2, C3, C9, C19	H2
2	31.4	1.50, 1.83	C3	H1, H3
3	75.7	3.54 (*m*)	-	H2, H4
4	41.9	2.20, 2.30	C1, C2, C3, C5, C6, C9	H3
5	139.0	-		
6	121.9	5.43 (*d*, 5)	C1, C4, C7, C8, C10	H7
7	27.2	1.80, 2.33	C5, C6, C9	H6, H8
8	35.7	1.80	C7, C9, C13, C14	H7, H9
9	43.1	1.30	C1, C10, C11, C19	H8, H11
10	37.2	-		
11	26.1	1.50, 1.78	C9, C10, C12, C13	H9, H12
12	71.4	4.67 (dd, 12, 4)	C1*, C13, C17, C18	H11
13	53.7	-		
14	85.7	-		
	-	4.27 (*s*, C14-O<u>H</u>)	C14, C15	
15	34.4	1.83, 2.00	C14, C16, C17	
16	24.3	2.00	C14, C20, C21	H17
17	57.2	3.16	C12, C13, C14, C16	H16
18	9.9	1.09 (*s*)	C12, C13, C14, C17,	
19	19.3	1.02 (*s*)	C1, C5, C9	

20	217.1	-		
21	33.1	2.23 (*s*)	C17, C20	
1*	167.7	-		
2*	128.7	-		
3*	137.9	6.96 (*q*, 7.5)	C1*, C2*, C5*	H4*
4*	14.50	1.87 (*d*, 7.5)	C1*, C2*, C3*	H3*
5*	12.1	1.91 (*s*)	C1*, C2*, C3*	

The monosaccharide **6a** was identified as D-cymarose ($[\alpha]_D$ +48°) by comparison with an authentic sample ($[\alpha]_D$ +52°) (Hayashi,1985).

The disaccharide **7a,** isolated from the hydrolysis studies, was identified as lilacinobiose ($[\alpha]_D$ +17°, lit. +24°) (Allgeier, 1968). The methylated derivative of lilacinobiose, methyl β-lilacinobiose **(7b)** ($[\alpha]_D$ +25°, lit. +28°) was isolated as a major product and the ^{1}H NMR of the disaccharide unit was identical to that described in the literature (Allgeier, 1968).

In light of the evidence described, the structure of the compound **1** was established as 3β-[β-D-thevetopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-cymaropyranosyloxy]-12β-tigloyloxy-14β-hydroxypregn-5-en-20-one.

The ^{1}H and ^{13}C data (Table 1) for compound **2** pointed to the presence in the molecule of three *O*-methylated 2,6-dideoxy sugars and a single *O*-methylated 6-deoxy sugar. The 2,6-dideoxy sugars were identified as cymarose moieties and the 6-deoxy sugar

was identified as a 3-*O*-methyl-6-deoxyallose moiety on the basis of the ^{1}H NMR data and fragmentation patterns in the mass spectrum. The β-linkages of the sugars of the glycoside **2** were revealed by the magnitude of the ^{1}H-^{1}H coupling constants, J = 9.6, 9.7, 7.8 and 9.7 Hz of the anomeric proton signals (atom numbers 1′,1″,1‴,1⁗, respectively). The sequence of the carbohydrate moieties was established by the 3-bond correlations obtained across the glycosidic bonds. These correlations were between the C-1′ anomeric proton (δ_H 4.78) and the C-3 resonance at δ_C 77.0, the C-1″ anomeric proton (δ_H 4.70) and the C-4′ resonance at δ_C 82.7 which confirmed that the first and second cymarose units were linked to each other, the C-1‴ anomeric proton (δ_H 4.23) and the C-4″ resonance at δ_C 82.5 that established that the 3-*O*-methyl-6-deoxyallose unit was attached to the second cymarose unit, and the C-1⁗ anomeric proton δ_H 4.78 and the C-4′ resonance at δ_C 81.7 which confirmed the terminal cymarose moiety. The aglycone was identified as 12-*O*-tigloyldigipurpurogenin II **(3**) by comparison with the ^{13}C NMR data of compound **1**.

Using a similar purification procedure, compounds **1** and **2** were also found in a dried sample of *H. pilifera.*

The purification and isolation of the trisaccharide glycoside **1** was guided by biological tests of the extracts and the fractions obtained from silica gel chromatography for appetite-suppressant activity on rats. A dose range-finding experiment was conducted on female rats at five doses using the trisaccharide glycoside **1**, with a control group receiving only the carrier substance. The test material was mixed with potato starch and dosage was orally for the first three consecutive days. The rats were monitored over an eight day period. The reduced

food intake (Table 4) in combination with the reduction in body mass gain and in some animals even a loss in body mass (Table 5), is strongly indicative of suppression of the appetite. Reduced food intake and reduced body mass gain was experienced even with the lowest dose group (6.25 mg/kg). No dose-related effect on water consumption was found. The treatment did not affect the health of the animals during the study period.

Table 4: Effect of compound **1** on food and water consumption in rats

Oral treatment (mg/kg/day)	Food consumption (g)							
	Day -7 to -1 (mean/day)	Day 1	Day 2	Day 3	Day 4	Day 5*	Day 6*	Day 7*
Vehicle	22.55	20.84	20.78	19.30	28.42	21.81	21.81	21.81
6.25	20.00	10.19	8.84	8.67	11.76	19.24	19.24	19.24
12.50	20.45	10.77	5.83	8.12	10.85	14.14	14.14	14.14
25.00	21.63	9.38	7.81	8.37	11.26	12.00	12.00	12.00
37.50	22.12	9.70	7.59	8.36	11.71	14.36	14.36	14.36
50.00	21.61	10.25	7.45	9.94	10.85	12.57	12.57	12.57
Oral treatment (mg/kg/day)	Water consumption (g)							
	Day -7 to -1 (mean/day)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Vehicle	29.01	26.90	27.70	27.98	27.53	35.38	35.38	35.38
6.25	23.26	56.15	55.06	38.22	30.44	30.76	30.76	30.76
12.50	24.55	45.72	34.35	26.97	42.57	37.71	37.71	37.71
25.00	33.24	21.53	33.57	32.16	37.39	34.12	34.12	34.12
37.50	24.96	32.92	23.79	24.63	39.19	38.78	38.78	38.78
50.00	30.73	49.30	30.96	38.53	41.99	45.94	45.94	45.94

* mean value calculated for 3 days (weekend)

Table 5: Effect of compound **1** on body mass in rats

Oral treatment (mg/kg/day)	Body mass (g)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 8
Vehicle	156.2	159.0	163.2	163.9	171.8	175.3
6.25	156.0	153.2	150.3	147.4	147.0	165.8
12.50	158.7	157.1	158.3	149.2	151.1	156.7
25.00	160.9	159.0	154.4	155.1	154.8	157.5

37.50	153.9	154.3	150.5	146.2	149.8	157.3
50.00	160.5	157.3	154.1	155.1	152.5	157.4

The glycoside was also evaluated against fenfluramine, a compound known to have appetite-suppressing properties. The test animals were dosed on the first three days and monitored over an eight day period. Compound **1** resulted in a reduction in food intake (Table 6) over the study period, with a concomitant overall decrease in body weight over the five day period (Table 7). Fenfluramine resulted in a small decrease in food intake, but an *increase* in body weight (though less than control group) over the same period of time.

Table 6: Effect of compound **1** and fenfluramine on food and water consumption in rats

Oral treatment (mg/kg/day)	Food consumption (g)								
	Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Vehicle	22.2 ±1.7	19.3 ±2.2	20.5 ±2.8	19.6 ±1.7	21.8 ±3.1	21.6 ±2.3	22.6 ±3.2	22.3 ±2.5	20.2 ±3.1
Compound 1 (30 mg/kg/day)	22.6 ±4.3	10.1** ±1.9	6.2*** ±1.1	7.7*** ±1.6	10.2*** ±2.6	12.5*** ±5.3	14.8** ±5.6	16.8* ±6.6	18.3 ±6.4
Fenfluramine (15 mg/kg/day)	23.0 ±3.8	7.4*** ±3.5	14.4** ±1.0	15.6*** ±0.6	22.3 ±1.5	23.1 ±2.3	24.0 ±4.0	22.0 ±1.6	20.6 ±1.8
	Water consumption (g)								
	Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Vehicle	28.4	28.6	26.8	31.3	25.3	28.4	25.8	30.3	29.9

	±1.6	±3.8	±3.5	±4.9	±2.8	±2.2	±3.8	±6.6	±6.3
Compound 1 (30 mg/kg/day)	30.4 ±7.4	27.3 ±5.4	27.8 ±7.5	30.0 ±4.1	27.9 ±10.0	30.2 ±11.5	26.0 ±7.8	30.5 ±8.6	34.5 ±10.0
Fenfluramine (15 mg/kg/day)	31.2 ±5.0	24.1 ±4.2	28.1 ±4.1	38.0 ±9.0	34.2* ±4.7	33.7 ±2.8	32.2 ±4.5	36.6 ±7.0	32.7 ±6.8

Significance of difference using Student's 't' test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Animals dosed on Days 1-3 only

Table 7: Effects of compound **1** and fenfluramine on body mass in rats

Oral treatment (mg/kg/day)	Group mean bodyweight (g)						
	Day -7	Day -5	Day -3	Day 1	Day 3	Day 5	Day 8
Vehicle	146 ±8.4	147 ±9.0	155 ±9.8	166 ±9.5	172 ±12.1	178 ±11.0	190 ±13.6
Compound 1 (30 mg/kg/day)	145 ±9.9	150 ±11.1	155 ±15.4	167 ±16.2	161 ±11.8	158** ±11.1	167* ±17.3
Fenfluramine (15 mg/kg/day)	143 ±9.5	143 ±9.9	151 ±10.2	161 ±13.3	157* ±10.5	165 ±11.8	177 ±11.3

Significance of difference using Student's 't' test: * $P < 0.05$; ** $P < 0.01$

Animals dosed on Days 1-3 only

These preliminary tests that were conducted on rats using the compound **1** indicated that the molecule has significant appetite-suppressant activity and also results in decreased body weights when consumed orally over a three day period.

1. Experimental

1.1 General

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Mass spectra were recorded on a Varian MAT 121 double-focussing mass spectrometer. HR-ESI-MS for compounds **1** and **2** were performed on a Waters Micromass LCT Premier TOF mass spectrometer with an electrospray source. Nuclear magnetic resonance spectra were recorded on a Bruker AM-300 spectrometer operating at 300.13 MHz for ^{1}H and 78.47 MHz for ^{13}C nuclei.

Purified fractions and reactions were monitored on Merck F254 pre-coated silica gel plates (0.25 mm thickness). Flash column chromatography was performed using Merck 230-400 mesh silica gel. Solvents used in chromatography were of technical grade and were distilled before use.

1.2 Plant material

Hoodia gordonii plant stems was collected from the Pella district in the Northern Cape. A voucher specimen (flowers) was deposited in the herbarium of the South African National Biodiversity Institute (SANBI), Pretoria.

1.3 Extraction and isolation

Plant stems (60 kg) were sliced and dried in an oven at 60 °C, milled to a coarse powder and extracted with a mixture of MeOH:CH_2Cl_2 (1:1). The resulting mixture was filtered and the organic solvent evaporated to give sticky material (8 kg). The extract was dissolved in MeOH–H_2O (9:1 v/v) and extracted with hexane followed by evaporation of the MeOH-H_2O layer until only H_2O remained. This was further partitioned between H_2O and CH_2Cl_2 followed by evaporation of the CH_2Cl_2 to dryness to give a dry solid residue (200 g).

The CH_2Cl_2 extract (200 g) obtained from the extraction of *H. gordonii* was subjected to column chromatography using solvents of increasing polarity from $CHCl_3$ to $CHCl_3$-MeOH (9:1 v/v). Appropriate fractions of the eluate were combined and further purified by several column chromatography purification steps using $CHCl_3$-MeOH (9:1 v/v) as the eluant. The trisaccharide **1** was isolated as a white solid and crystallised from Me_2CO-hexane m.p. 135-140 °C, (8 g), HR-ESI-MS: 901.4943 (901.4925 calcd for $[C_{47}H_{74}O_{15}+Na]$) . The ^{1}H and ^{13}C NMR data are collated inTables 1 and 2, respectively.

The tetrasaccharide **2** was also isolated as a white solid and crystallised from Me_2CO-hexane m.p. 127-132 °C (1.1 g), HR-ESI-MS: 1045.5631 (1045.5712 calcd for $[C_{54}H_{86}O_{18}+Na]$).The ^{1}H and ^{13}C NMR data are collated in Tables 1 and 2, respectively.

*Acid hydrolysis of steroidal trisaccharide **1***

A solution of the trisaccharide **1** (80 mg) in MeOH (15 ml) was treated with H_2SO_4 (0.2 M, 5 ml) and the mixture was allowed to stand at 70 °C for 30 min, after which it was extracted with Et_2O (50 ml). The Et_2O layer was dried ($MgSO_4$), filtered and evaporated. The residue was separated by column chromatography on silica gel, using $CHCl_3$-MeOH (9.5:0.5) as the eluant to afford 12-O-tigloyldigipurpurogenin II **(3)** (30 mg) and the monoglycoside **4** (8 mg). The aq. layer from above was neutralised with $Ba(OH)_2$ (1%). The ppt. was removed by filtration and the filtrate evaporated to dryness. The mixture of compounds was separated by column chromatography on silica gel using EtOAc-toluene (1:1) as the eluant to afford methyl ß-D-cymaropyranoside **(6b)** (15 mg) and methyl ß-lilacinobioside **(7b)** (18 mg).

Methyl β-D-cymaropyranoside ***(6b)***

^{13}C NMR ($CDCl_3$) δ_c 18.1Q (C-6), 33.4T (C-2), 56.2Q (methoxy), 56.9Q (methoxy), 72.0D (C-5), 77.2D (C-4), 78.5D (C-3), 98.7D (C-1).

-Methy β-lilacinobioside ***(7b)***

^{1}H NMR ($CDCl_3$) δ_H 1.29 (3H, d, *J* = 6.3 Hz, 6-H), 1.29 (3H, d, *J* 6.3 Hz, 6′-H), 1.59 (1H, ddd, *J* 13.5, 9.1, 2.5 Hz, 2_{ax}-H), 2.16 (1H, ddd, *J* 13.5, 4.5, 2.1 Hz, 2_{eq}-H), 3.09 (1H, dd, ^{3}*J* 8.0, 2.9 Hz, 3′-H) 3.17 (1H, dd, *J* 9.7, 2.9, 1.1 Hz, 4′-H), 3.29 (1H, dd, *J* 9.4, 3.0 Hz, 4-H), 3.36 (1H, dd, *J* 9.7, 6.3 Hz, 5′-H), 3.41[a] (3H, s, 1-OCH_3), 3.45[a] (3H, s, 3-OCH_3), 3.51 (1H, dd, *J* 8.0, 7.7 Hz, 2′-H), 3.64 (3H, s, 3′-OCH_3) 3.79 (1H, m, 3-H), 3.92 (1H, dd, *J* 9.4, 6.3 Hz, 5-H), 4.31 (1H, d, *J* 7.0 Hz, 1′-H), 4.64 (1H, dd, *J* 9.1, 2.1 Hz, 1-H).

[a] Assignments may be interchanged

12-*O-Tigloyldigipurpurogenin II* ***(3)*** – Found: M^+ 430.275, $C_{26}H_{38}O_5$ requires: *M* 430.279, see Table 3 for NMR data.

3-O-(β-D-Cymaropyranosyl)-12-O-tigloyldigipurpurogenin II ***(4)***

(Found M^+ 558.361, $C_{33}H_{50}O_7$ requires: M 558.365), ^{1}H NMR ($CDCl_3$) δ_H 0.98 (3H, s, 19-H), 1.03 (3H, s, 18-H), 1.25 (3H, d, *J* 6.2 Hz, 6′-H), 1.80 (3H, dd, *J* 6.9, 1.1 Hz, 4*-H), 1.88 (3H, dd, *J* 1.5, 1.1 Hz, 5*-H), 2.17 (3H, s, 21-H), 3.19 (1H, dd, *J* 9.2, 2.5 Hz, 4′-H), 3.39 (3H, s, OCH_3), 3.48-3.62 (3H, m, 3-H, 17-H, 5′-H), 4.18 (1H, dd, *J* 5.6, 3.5 Hz, 3′-H), 4.24 (1H, s, OH), 4.29 (1H, s, OH), 4.61 (1H, dd, *J* 11.4, 4.0 Hz, 12-H), 4.75 (1H, dd, *J* 9.6, 1.8 Hz, 1′-H), 5.39 (1H, m, 5-H), 6.89 (1H, dq, *J* 7.1, 1.5

Hz, 3*-H); ^{13}C NMR ($CDCl_3$) δ_c, 9.9Q (C-18), 12.1Q (C-5*), 14.0Q (C-4*), 18.3Q (C-6′), 19.3Q (C-19), 24.4T (C-16), 26.1T (C-11), 27.4T (C-7), 29.6T (C-2), 33.1Q (C-20), 34.1T (C-15), 3.4T (C-2′), 35.7D (C-8), 37.1S (C-10), 37.2T (C-1), 38.6T (C-4), 43.0D (C-9), 53.6S (C-13), 53.7Q (methoxy), 57.2D (C-17), 68.1D (C-5′), 70.7D (D-4′), 75.9D (C-12), 77.4D (C-3), 77.5D (C-3′), 85.7S (C-14), 95.5D (C-1′), 122.0D (C-6), 128.8S (C-2*), 137.8D (C-3*), 139.2S (C-5), 167.7S (C-1*), 217.0S (C-20). *refers to the tigloate group atoms.

*Digipurpurogenin II **(5a)***

12-*O*-Tigloyldigipurpurogenin II **(3)** (70 mg) was stirred in a solution of 5% KOH in MeOH (2 ml) for 3 h. H_2O (15 ml) was added, and the reaction mixture was extracted with Et_2O (3 x 20 ml). The Et_2O layer was dried with $MgSO_4$, filtered and evaporated. The epimers, **5a** and **5b** were separated by column chromatography on silica gel using $CHCl_3$-MeOH (9:1) as the eluant to afford digipurpurogenin II **(5a)** (R_f 0.35) (12 mg) m.p. 222-227 °C (Me_2CO), (lit. 226-223 °C) (Mitsuhashi, 1965), (Found M^+ 348.231, C; 72.29, 9.20% $C_{21}H_{32}O_4$ requires: C, 72.38; 9.26%, *M* 348.236), and *iso*-digipurpurogenin II **(5b)** (4 mg), m.p. 185-192 °C (Me_2CO), (lit. 184-196°) (Mitsuhashi, 1965).

*Digipurpurogenin II **(5a)***

^{1}H NMR ($CDCl_3$) δ_H 0.97 (3H, s, 19-H), 1.20 (3H, s, 18-H), 2.25 (3H, s, 21-H), 3.32 (1H, dd, *J* 11.1, 3.9 Hz, 12-H), 3.47 (1H, s, OH), 3.53 (1H, m, 3-H), 3.60 (1H, dd, *J* 9.5, 9.5 Hz, 17-H), 4.30 (1H, s, OH), 5.39 (1H, m, 5-H).

*iso-Digipurpurogenin II **(5b)***

^{1}H NMR ($CDCl_3$) δ_H 0.98 (3H, s, 19-H), 1.52 (3H, s, 18-H), 2.24 (3H, s, 21-H), 3.33 (1H, dd, *J* 9.4 Hz, 17-H), 3.49 (1H, m, 3-H), 3.63 (1H, dd, *J* 11.1, 3.9 Hz, 12-H), 3.70 (1H, s, OH), 4.32 (1H, s, OH), 5.38 (1H, m, 5-H).

1.4 Biological assay

General procedure

Female Wistar rats (body weight 110 to 150 g) and approximately 6 - 7 weeks of age were housed individually in suspended metal cages with wire grid floors. Upon arrival, the rats were checked for abnormalities and overt signs of ill health; all rats appeared normal. The rats had access to tap water and were fed a standard certified laboratory rodent diet (RM1(E)SQC), *ad libitum*. Lighting in the animal room was controlled to give 12 hours light (0700 to 1900 hours) and 12 hours dark each day. The room temperature and relative humidity controls were set at 21 ℃ ± 3 ℃ and 55% ± 15%, respectively. Room temperature and humidity were recorded continuously in the holding room and did not deviate outside the stated limits. The animals were randomised into groups according to bodyweight using random numbers tables so that the group mean bodyweights were approximately equal. Including the day of randomisation, there was a further 7 days of acclimatisation prior to dosing during which time food and water consumption were recorded.

Dose-range experiment

Each treatment group consisted of 3 animals, with 6 animals in the control group. The test article was mixed with prepared potato starch (Kyron Labs) immediately prior to dosing on each day. A range of five doses of compound **1** (6.25, 12.50, 25.00, 37.50 and 50.00 mg/kg bodyweight) were administered, with a control group receiving only

the carrier substance, by oral gavage once daily on Day 1, 2 and 3 of the study using a constant dose volume of 1 ml/kg bodyweight. Bodyweights, food consumption (food hopper weight) and water consumption (bottle weight) were recorded daily from day -8 until the termination of the study. The effects on food and water consumption are given in Table 4 and the effect on bodyweight is given in Table 5.

*Comparison between steroid glycoside **1** and fenfluramine*

Each treatment group consisted of 6 animals. Compound **1**, formulated at 30 mg/kg bodyweight, and fenfluramine formulated at 15 mg/kg bodyweight, and vehicle alone were administered by gavage once daily on Day 1, 2 and 3 of the study using a constant dose volume of 1 ml/kg bodyweight. Bodyweights, food consumption (food hopper weight) and water consumption (bottle weight) were recorded daily from day -8 until the termination of the study. The effects on food and water consumption are given in Table 6 and the effect on bodyweight is given in Table 7. Significance of difference was performed using students “t” test.

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