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Phytochemistry 68 (2007) 2545–2553
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

An appetite suppressant from *Hoodia* species

Fanie R. van Heerden [a,*], R. Marthinus Horak [b], Vinesh J. Maharaj [c,*], Robert Vleggaar [d], Jeremiah V. Senabe [c], Philip J. Gunning [e]

[a] *School of Chemistry, University of KwaZulu-Natal, Private Bag X01, Scottsville 3209, South Africa*
[b] *TTSI, CSIR, RSA, P.O. Box 395, Pretoria 0001, South Africa*
[c] *Biosciences, CSIR, RSA, P.O. Box 395, Pretoria 0001, South Africa*
[d] *Department of Chemistry, University of Pretoria, Pretoria 0002, South Africa*
[e] *Phytopharm plc, Corpus Christi House, 9 West Street, Godmanchester, Cambridgeshire PE29 2HY, United Kingdom*

Received 18 December 2006; received in revised form 7 May 2007
Available online 2 July 2007

Abstract

Studies conducted at the Council for Scientific and Industrial Research (CSIR, South Africa) identified extracts from *Hoodia* species, in particular *Hoodia pilifera* and *Hoodia gordonii*, as possessing appetite suppressing properties. Two pregnane glycosides were isolated by fractionation of the dried stems of *H. 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-thevetopyranosyl-(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 *H. pilifera*. Compound **1** was tested for its appetite suppressant properties in rats by oral gavage at 6.25–50 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

1. Introduction

Indigenous plants have always formed part of the diet of communities in rural areas. During the early sixties, an investigation was launched at the CSIR to determine the nutritional value and also any possible long-term toxic effects of 'food from the veld'. The National Food Research Institute of CSIR, one of the institutes now incorporated into the CSIR Biosciences unit, screened more than 1000 species of wild South African plants known to be used as indigenous 'bush foods', including plants of the genus *Hoodia*.

During 1983, 20 years after the initial research started at CSIR, a detailed investigation of the *Hoodia pilifera* plant was undertaken, with the finding that extracts of *H. pilifera* had appetite suppressing effects in rats. This research was extended into the investigation of other *Hoodia* species, including *H. gordonii*, resulting in a patentable invention (Van Heerden et al., 1998). Subsequently other workers have confirmed the appetite-suppressing effect of extracts obtained from *Hoodia* species (Tulp et al., 2001).

H. 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.

* Corresponding authors. Tel.: +27 12 841 3295; fax: +27 12 841 3295.
E-mail address: vmaharaj@csir.co.za (V.J. Maharaj).

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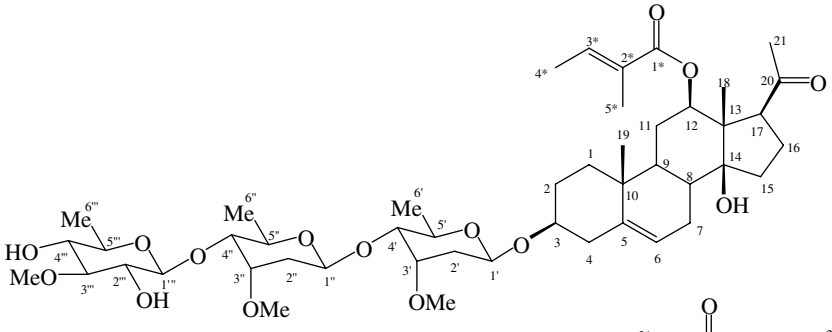
doi:10.1016/j.phytochem.2007.05.022

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 and Sloane, 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 *H. 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 and Sloane, 1937). The plant has a more bitter taste that is persistent and spreads around the mouth (Bruyns, 1993).

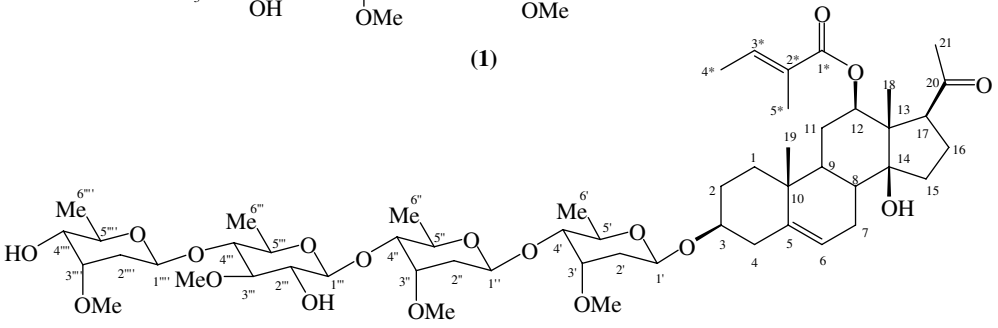
The present paper describes the isolation, structure elucidation and biological activity of two compounds extracted from *H. gordonii* and *H. pilifera*.

2. Results and discussion

Extraction of the dried milled aerial parts of *H. gordonii* (60 kg) with a methanol–dichloromethane solution, followed by solvent–solvent partitioning and extensive column chromatography, led to the isolation of compounds **1** (8 g) and **2** (1.1 g).



(1)



(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 11 methyl, 9 methylene, 20 methine and 7 quaternary carbon signals and 54 resonances observed for glycoside **2** consisting of 13 methyl, 10 methylene, 24 methine and 7 quaternary atoms.

The ^1H and ^{13}C data (Table 1) 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 ^1H–^1H coupling constants, $J = 9.6$, 9.6 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 et al., 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 et al., 1993). The methyl groups of the 6-deoxy sugars were at δ_H 1.31, 1.27, 1.21 and methoxy groups of the sugars were at δ_H 3.44, 3.42, 3.65. The remainder of the signals in the ^1H NMR spectrum of the glycoside exhibited complex structure. In this instance, the proton–proton connectivity pattern and chemical shift values could be obtained from ^1H–^1H COSY experiments.

Sequence information of the sugars of the glycoside and the substitution pattern of the aglycone were deduced from HMBC experiments in which three-bond correlations were observed across the glycosidic bonds. These correlations were between the C-1″ anomeric proton (δ_H 4.75) and the

Table 1
NMR data (500 MHz, $CDCl_3$) of glycosides **1** and **2**

	1			**2**		
Carbon	δ_C	δ_H[#]	HMQC (H to C)	δ_C	δ_H[#]	HMQC (H to C)
1	37.2	ax: 1.08 (td, *J* 13.7, 3.8)		37.2	1.08 (td, *J* 13.5, 3.7)	
		eq: 1.80			1.79	
2	29.5	1.52		29.4	1.54	
		1.92			1.92	
3	77.5	3.52 (m)	C-1′	77.46[a]	3.53 (m)	C-1′
4	38.6	2.18	C-3, 6	38.6	2.16	
		2.36			2.36	
5	139.0			139.0		
6	122.0	5.41 (br. s)	C-4, 10	122.0	5.40 (br. s)	C-4, 8, 10
7	27.3	1.80		27.3	1.81	
		2.31			2.31	
8	35.7	1.76	C-11	35.7	1.78	
9	43.0	1.27		43.0	1.27	
10	37.1			37.1		
11	26.1	ax: 1.47 (q, *J* 12.4)	C-9, 12, 17	26.1	1.47 (q, *J* 12.6)	C-9, 12, 17
		eq: 1.77	C-9		1.79	
12	75.9	4.63 (dd, *J* 12.0, 4.5)	C-17, 18, 1*	75.9	4.63 (dd, *J* 12.0, 4.4)	C-1*, 13, 17, 18
13	53.7			53.7		
14	85.7			85.7		
15	34.4	1.98		34.4	1.98	
		1.86	C-14		1.86	
16	24.4	1.97	C-20	24.4	1.97	C-20
		1.97			1.97	
17	57.2	3.13 (d, *J* 8.8, 5.0)	C-13	57.2	3.13 (dd, *J* 8.8, 1.5)	C-20
18	9.9	1.06 (s)	C-12, 13, 14, 17	9.9	1.05 (s)	C-12, 13, 14, 17
19	19.3	0.98 (s)	C-1, 5, 9, 10,	19.3	0.98 (s)	C-1, 5, 9, 10
20	217.0			217.0		
21	33.1	2.19 (s)	C-17, 20	33.1	2.19 (s)	C-17,20
Cymarose-1						
1′	95.9	4.84 (dd, *J* 9.6, 2.0)	C-3	95.8	4.84 (dd, *J* 9.7, 1.8)	C-3
2′	35.5	2_{ax} 1.56 (ddd, *J* 13.8, 9.6, 2.5)	C-1′	35.5	1.56 (ddd, J 13.5, 9.7, 2.5)	
		2_{eq} 2.08 (ddd, *J* 13.8, 3.6, 2.0)			2.08 (ddd, *J* 13.5, 3.4, 2.0)	
3′	77.0	3.80 (q, *J* 3.0)		77.04[b]	3.80 (q, 2.9)	
4′	82.6	3.20 (dd, *J* 9.8, 2.9)	C-1″, 5′, 6′	82.5	3.20	C-4″
5′	68.5	3.84 (dq, *J*9.6, 6.2)		68.5	3.83 (dq, *J* 9.6, 6.2)	
6′	18.2	1.21 (d, *J* 6.1)		18.3[c]	1.20 (d, *J* 6.2)	
OMe	58.0	3.44 (s)	C-3′	58.1[d]	3.42[a] (s)	C-3′
Cymarose-2						
1″	99.6	4.75 (dd, *J* 9.6, 2.0)	C-4′	99.6	4.75 (dd, *J* 9.7, 1.8)	
2″	35.1	2_{ax} 1.65 (ddd, *J* 14.0, 9.6, 2.6)	C-1″	35.3	1.64 (ddd, *J* 14.0, 9.7, 2.5)	
		2_{eq} 2.15 (ddd, *J* 14.0, 3.1, 2.0)			2.13 (ddd, *J*, 14.0, 3.4, 1.8)	
3″	76.9	3.78 (q, *J* 3.0)		77.00[b]	3.75 (q, *J* 3.0)	
4″	82.7	3.26 (dd, *J* 9.6, 3.0)	C-1‴, 5″, 6″	82.7	3.25 (dd, *J* 9.7, 2.9)	C-1‴
5″	68.3	3.90 (dq, *J* 9.6, 6.2)		68.3	3.90 (dq, *J* 9.7, 6.2)	
6″	18.4	1.27 (d, *J* 6.3)		18.2[d]	1.27 (d, *J* 6.2)	
OMe	57.9	3.42 (s)	C-3″	57.9[d]	3.43[a] (s)	C-3″
Thevetose-3						
1‴	104.3	4.30 (d, *J* 7.7)	C-4″	104.1	4.28 (d, *J* 7.8)	C-4″
2‴	74.7	3.50 (dd, *J* 9.0, 7.9)	C-1‴, 3‴	74.0	3.47 (t, *J* 8.4)	C-1‴, 3‴
3‴	85.2	3.10 (t, *J* 9.0)	C-2‴, 4‴	84.0	3.22 (t, *J* 8.7)	
4‴	74.6	3.18 (t, *J* 9.0)	C-3‴, 5‴, 6‴	81.7	3.26 (t, *J* 8.9)	C-1‴′
5‴	71.6	3.36 (dq, *J* 9.0, 6.3)		71.2	3.37 (dq, *J* 9.0, 6.2)	C-4‴
6‴	17.8	1.31 (d, *J* 6.3)	C-4‴, C-5‴	18.2[c]	1.27 (d, *J* 6.2)	
OMe	60.7	3.65 (s)	C-3‴	60.0	3.61 (s)	C-3‴
Cymarose-4						
1‴′				98.3	4.82 (dd, *J* 9.6, 1.9)	C-4‴
2‴′				34.4	1.55 (ddd, *J* 14.0, 9.6, 2.5) 2.25 (ddd, *J* 14.0, 2.8, 2.0)	
3‴′				77.43[a]	3.62 (q, *J* 3.2)	

(*continued on next page*)

Table 1 (*continued*)

Carbon	1			2		
	δ_C	δ_H[#]	HMQC (H to C)	δ_C	δ_H[#]	HMQC (H to C)
4′′′′				72.4	3.19 (dd, *J* 9.6, 3.5)	
5′′′′				71.1	3.59(dq, *J* 9.6, 6.2)	
6′′′′				18.0[c]	1.29 (d, *J* 6.2)	
OMe				57.1[d]	3.42[a] (s)	C-3′′′′
Tigloyl						
1*	167.7			167.7		
2*	128.7			128.7		
3*	137.8	6.92 (qq, *J* 7.0 and 1.3)	C-1*,4*,5*	137.8	6.92 (qq, 7.0, 1.4)	C-1*,4*,5*
4*	14.5	1.83 (br. d, *J* 7.2)	C-2*, 3*	14.5	1.83 (dd, 7.0, 0.9	C-1*, 2*, 3*
5*	12.1	1.88 (br.s)	C-1, 2*, 3*	12.1	1.88 (br. s)	C-1*, 2*, 3*

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

[#] When splitting pattern and coupling constants are not given, extensive overlap of signals has prevented the assignment thereof.

[*] Refers to the tigloyl atoms.

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 4.30) and the C-4′′ resonance at δ_C 82.7 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 ($J = 1.3$ Hz) was observed between the vinylic proton which resonates at δ_H 6.92 and the protons of the methyl group located on a sp^2 carbon atom (δ_H 1.88).

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 4.63. The broadness of the resonance for H-3 and the coupling constants of $J = 12.0$ and 4.5 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 0.98, 1.06 and 2.19 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 substitution pattern on the aglycone: (i) correlation between the C-12 proton (δ_H 4.63) and the singlet resonance (δ_C 167.7) 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 95.9) 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.

The aglycone moiety was identified as 12-*O*-tigloyldigipurpurogenin II (**3**) based on NMR analysis (Table 2). 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 as described previously (Rubin, 1963). A similar epimeric

Table 2
^{1}H and ^{13}C NMR data (300 MHz, $CDCl_3$) for aglycone **3**

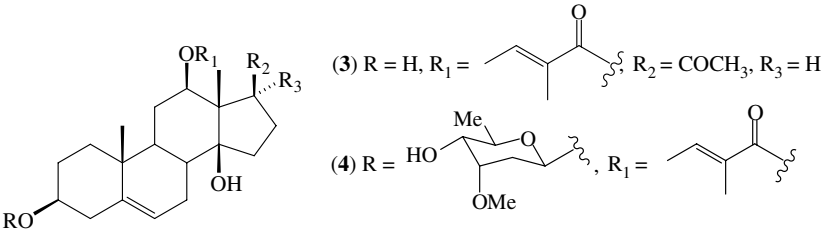
Carbon	δ_C	δ_H	^{1}H–^{13}C connectivity (HMBC)	^{1}H–^{1}H connectivity (COSY)
1	36.8	1.12, 1.80	C-2 ,3, 9, 19	H2
2	31.4	1.50, 1.83	C-3	H1, H3
3	75.7	3.54 (m)	–	H2, H4
4	41.9	2.20, 2.30	C-1, 2, 3, 5, 6, 9	H3
5	139.0	–		
6	121.9	5.43 (d, *J* 5.0)	C-1, 4, 7, 8, 10	H7
7	27.2	1.80, 2.33	C-5, 6, 9	H6, H8
8	35.7	1.80	C-7, 9, 13, 14	H7, H9
9	43.1	1.30	C-1, 10, 11, 19	H8, H11
10	37.2	–		
11	26.1	1.50, 1.78	C-9, 10, 12, 13	H9, H12
12	71.4	4.67 (dd, *J* 12.0, 4.0)	C-1*, 13, 17, 18	H11
13	53.7	–		
14	85.7	–		
	–	4.27 (s, C14-OH)	C-14, 15	
15	34.4	1.83, 2.00	C-14, 16, 17	
16	24.3	2.00	C-14, 20, 21	H17
17	57.2	3.16	C-12, 13, 14, 16	H16
18	9.9	1.09 (s)	C-12, 13, 14, 17,	
19	19.3	1.02 (s)	C-1, 5, 9	
20	217.1	–		
21	33.1	2.23 (s)	C-17, 20	
1*	167.7	–		
2*	128.7	–		
3*	137.9	6.96 (q, *J* 7.5)	C-1*, 2*, 5*	H4*
4*	14.50	1.87 (d, *J* 7.5)	C-1*, 2*, 3*	H3*
5*	12.1	1.91 (s)	C-1*, 2*, 3*	

[*] Refers to the tigloyl atoms.

ratio was also obtained during the base hydrolysis of 12-*O*-benzoyldigipupurogenin II (Mitsuhashi and Nomura, 1965). The epimers were separated by silica flash chromatography and compared favourably with the published data for digipurpurogenin II (**5a**) (Tschesche et al., 1961).

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

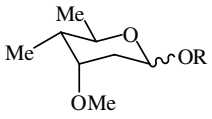
The disaccharide **7a**, isolated from the hydrolysis studies, was identified as lilacinobiose ($[\alpha]_D + 17$, lit. +24) (Allgeier, 1968). The methylated derivative of lilacinobiose, methyl β-lilacinobiose (**7b**) ($[\alpha]_D + 25$, lit. +28) was isolated as a major product and the ^{1}H NMR of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



$R_2 = COCH_3$, $R_3 = H$

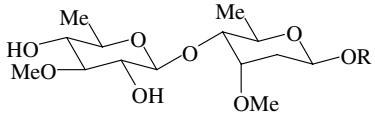
(**5a**) R = H, R_1 = H, $R_2 = COCH_3$, R_3 = H

(**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

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-cymaropyranosyl-oxy]-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 thevetose 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, $J = 9.7$, 9.7, 7.8 and 9.6 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 4.84) and the C-3 resonance at δ_C 77.5, the C-1″ anomeric proton (δ_H 4.75) and the C-4′ resonance at δ_C 82.5 which confirmed that the first and second cymarose units were linked to each other, the C-1‴ anomeric proton (δ_H 4.28) and the C-4″ resonance at δ_C 82.7 that established that the thevetose unit was attached to the second cymarose unit, and the C-1⁗ anomeric proton δ_H 4.82 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 3) in combination with the reduction in body mass gain and in some animals even a loss in body mass (Table 4), 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.

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 5) over the study period, with a concomitant overall decrease in body mass gain over the five day period (Table 6). Fenfluramine resulted in a small decrease in food intake, but an *increase* in body

Table 3
Effect of compound **1** on food and water consumption in rats

Oral treatment (mg/kg/day)[a]	Food consumption (g)							
	Day -7 to -1 (mean/day)	Day 1	Day 2	Day 3	Day 4	Day 5[b]	Day 6[b]	Day 7[b]
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
	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

[a] Rats received three treatments (day 1, 2 and 3)
[b] Mean value calculated for 3 days (weekend).

Table 4
Effect of compound **1** on body mass in rats

Oral treatment (mg/kg/day)[a]	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

[a] Rats received three treatments (day 1, 2 and 3).

Table 5
Effect of compound **1** and fenfluramine on food and water consumption in rats

Oral treatment (mg/kg/day)[a]	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*** pm 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 ± 1.6	28.6 ± 3.8	26.8 ± 3.5	31.3 ± 4.9	25.3 ± 2.8	28.4 ± 2.2	25.8 ± 3.8	30.3 ± 6.6	29.9 ± 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': $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.
[a] Rats received three treatments (day 1, 2 and 3).

mass gain relative to compound **1** (though less than the control group) over the same period of time.

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. A possible mode of action of compound **1** has been reported (MacLean and Luo, 2004).

Table 6
Effects of compound **1** and fenfluramine on body mass in rats

Oral treatment (mg/kg/day)[a]	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': $^{*}P < 0.05$; $^{**}P < 0.01$.
[a] Rats received three treatments (day 1, 2 and 3).

3. Experimental

3.1. 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 Varian Inova 500 spectrometer operating at 499.98 for ^{1}H and 125.73 MHz for ^{13}C nuclei or a Bruker AM-300 spectrometer operating at 300.13 MHz for ^{1}H and 75.47 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.

3.2. Plant material

H. gordonii plant stems was collected from the Pella district in the Northern Cape. A voucher specimen (PRE 797004) was deposited in the herbarium of the South African National Biodiversity Institute (SANBI), Pretoria.

3.3. 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 (8 g) m.p. 135–140 °C, UV (CH_3CN) λ_{max} 220 nm ($\log\varepsilon = 4.06$), HR–ESI–MS *m*/*z*: 901.4943 (901.4925 calcd for [$C_{47}H_{74}O_{15}$+Na]). The ^{1}H and ^{13}C NMR data are collated in Table 1.

The tetrasaccharide **2** was also isolated as a white solid and crystallised from Me_2CO–hexane m.p. 127–132 °C (1.1 g), UV (CH_3CN) λ_{max} 220 nm ($\log\varepsilon = 4.08$), HR-ESI-MS *m*/*z*: 1045.5631 (1045.5712 calcd. for [$C_{54}H_{86}O_{18}$+Na]). The ^{1}H and ^{13}C NMR data are collated in Table 1.

3.4. 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 aqueous layer from above was neutralised with $Ba(OH)_2$ (1%). The precipitate 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 β-D-cymaropyranoside (**6b**) (15 mg) and methyl β-lilacinobioside (**7b**) (18 mg).

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

^{13}C NMR (75 MHz, $CDCl_3$)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).

3.6. Methy β-lilacinobioside (*7b*)

^{1}H NMR (300 MHz, $CDCl_3$)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, t, *J*8.9 Hz, 3′-H) 3.17 (1H, t, *J* 9.2 Hz, 4′-H), 3.29 (1H, dd, *J* 9.4, 3.0 Hz, 4-H), 3.36 (1H, dq, *J*9.7, 6.3 Hz, 5′-H), 3.38[1] (3H, s, 1-OCH_3), 3.42[1] (3H, s,

[1] Assignments may be interchanged.

3-OCH_3), 3.49 (1H, dd, *J* 9.0, 7.8 Hz, 2′-H), 3.64 (3H, s, 3′-OCH_3) 3.79 (1H, m, 3-H), 3.92 (1H, dq, *J* 9.4, 6.3 Hz, 5-H), 4.31 (1H, d, *J* 7.8 Hz, 1′-H), 4.64 (1H, dd, *J* 9.1, 2.1 Hz, 1-H).

3.7. 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.

3.8. 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 (300 MHz, $CDCl_3$)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, dq, *J* 6.9, 1.1 Hz, 4*-H), 1.88 (3H, dq, *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, 6-H), 6.89 (1 H, 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), 35.4T (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 (C-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).

3.9. 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 × 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 and Nomura, 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 and Nomura, 1965).

3.10. Digipurpurogenin II (5a)

^{1}H NMR (300 MHz, $CDCl_3$)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, 6-H).

3.11. Iso-digipurpurogenin II (5b)

^{1}H NMR (300 MHz, $CDCl_3$)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, 6-H).

3.12. Biological assay

3.12.1. General procedure

Female Wistar rats (body weight 110–150 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 h light (0700–1900 h) and 12 h dark each day. The room temperature and relative humidity controls were set at 21 ± 3 °C 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.

3.12.2. 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 3 and the effect on bodyweight is given in Table 4.

3.12.3. 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 5 and the effect on bodyweight is given in Table 6.

Significance of difference was performed using students "*t*-test".

Acknowledgement

This work was financially supported by the Council for Scientific and Industrial Research.

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