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Bufadienolides from bulbs of *Urginea lydenburgensis* (Hyacinthaceae: Urgineoideae)

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Abstract

Bulbs of the ethnomedicinal hyacinthac *Urginea lydenburgensis* have yielded two bufadienolides, 16β-acetoxy-3β,14β-dihydroxy-19-formyl-bufa-4,20,22-trienolide (scillicyanosidin) and 4β,8β,11α,14β-tetrahydroxybufa-5,20,22-trienolide-12-one, 2α,3β-*O*-4,6-dideoxy-L-glucose (lydenburgenin).

Keywords: Amaryllidaceae; *Urginea lydenburgensis*; Bufadienolides; 16β-Acetoxy-3β,14β-dihydroxy-19-formyl-bufa-4,20,22-trienolide (scillicyanosidin); 4β,8β,11α,14β-Tetrahydroxybufa-5,20,22-trienolide-12-one, 2α,3β-*O*-4,6-dideoxy-L-glucose (lydenburgenin)

1. Introduction

Urginea lydenburgensis R.A. Dyer of the subfamily Urgineoideae (family Hyacinthaceae) occurs in the Mpumalanga Province of South Africa, particularly the Lydenburg District (Dyer, 1942). Although it is generally accepted that the genus *Urginea* Steinh. should be placed in synonymy under *Drimia* Jacq. (Jessop, 1977; Stedje, 1987; Manning and Goldblatt, 2003), there is presently little consensus on species boundaries in *Drimia* s.l., particularly in the summer-rainfall region of South Africa. *U. lydenburgensis* is one such taxon that has been variously accepted (Reid, 1993), or alternatively synonymised under *Drimia delagoensis* (Bak.) Jessop (Jessop, 1977). However, as *U. lydenburgensis* has not yet been formally transferred to *Drimia*, this current phytochemical report employs the basionym. *U. lydenburgensis* is a highly toxic species responsible for stock losses, which is how it first attracted the attention of toxicologists (Van der Walt and Steyn, 1939) and subsequently the taxonomist (the species' author) from whom a plant identification was requested (Dyer, 1942). Fresh bulbs in the pre-flowering state proved fatal when administered to sheep and rabbits; sheep exhibited tympanites, apathy, anorexy, diuresis and severe diarrhoea (Van der Walt and Steyn, 1939), symptoms consistent with cardiotoxicosis (Kellerman et al., 1988).

The subfamily Urgineoideae is phytochemically characterised by the presence of bufadienolides (Speta, 1998; Pohl et al., 2000). Although bufadienolides show typical digitalis-like activity, the therapeutic usefulness of these compounds is unfortunately impaired by side effects which include severe gastric irritation (Sapeika, 1951; Majinda et al., 1997). The sap of leaves and bulbs of *Drimia* (syn. *Urginea*) species are irritating to the skin; some species produce such marked topical stinging and itching effects that they are used by young Xhosa boys during pain-endurance games. Bufadienolide-containing plants are also used as anthelmintics, for bronchial asthma, heart conditions, fevers and during pregnancy

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(Hutchings and Terblanche, 1989). Ethnomedicinal plant traders in Nelspruit (Mpumalanga Province) sell *U. lydenburgensis* under the names *masi xabane* and *isiklenama*, for internal use in treating asthma and itching of the skin. Vendors also recommend that the plant be administered internally in conjunction with either *Drimia altissima* (L.f.) Ker Gawl. or *Urginea epigea* R.A. Dyer, to respectively relieve asthma, and "blood impurities". A Swazi traditional medical practitioner from Nelspruit reportedly alleviated "body pains" (possibly rheumatism) by washing the whole body with a solution prepared by soaking the finely chopped bulbs in water. Patients reported experiencing a bracing, stinging sensation (*J. Onderstall 381*, PRE), consistent with the presence of bufadienolides. This information represents the first published account of *U. lydenburgensis* as an ethnomedicinal subject. The Swazi practitioner further recognised the poisonous character of the bulb and recommended that it should not be eaten.

Two novel bufadienolides were isolated from *U. lydenburgensis*.

2. Results and discussion

Compounds **1** and **2** were isolated from the dichloromethane extract of *U. lydenburgensis*. Use was made of ^{1}H, ^{13}C, HSQC, HMBC, COSY, TOCSY and NOESY spectra to determine the structures of compounds **1** and **2** and to assign ^{1}H and ^{13}C NMR resonances (Tables 1 and 2). The molecular formula, $C_{26}H_{32}O_7$, was indicated by the positive-ion FAB-MS of compound **1** (*m/z* 457 $(M+H]^{+}$). Compound **1** was characterised as a bufadienolide by the proton and carbon resonances of the δ-pyrone ring. Two doublets at δ7.21 ($J = 2.5$ Hz) and δ6.17 ($J = 9.8$ Hz) were seen to be coupled to a double doublet at δ7.95 ($J = 2.5$, 9.8 Hz) in the COSY spectrum and were assigned as H-21, H-23 and H-22, respectively. The HMBC spectrum showed correlations between the H-21 and H-22 resonances and a resonance at δ56.8 (CH) ascribed to C-17. The singlet integrating to three protons at δ0.77 showed correlations to the C-17 resonance in the HMBC spectrum and was assigned to 3H-18. A quaternary carbon resonance in the δ80–85 region of the ^{13}C NMR spectrum

Table 1
^{1}H, ^{13}C, HMBC, NOESY and COSY NMR data for compound **1**

	^{1}H NMR data for compound **1**[a]	^{13}C NMR data for compound **1**[b]	HMBC correlations H → C	NOESY correlations	COSY Correlations
1	2.34	32.6 (CH_2)	5, 10, 19	19, 4	2α, 2β
2	2.08α	29.5 (CH_2)	3, 4, 10	–	1, 3
	1.33β		3, 19	3, 19	1, 3
3	4.15 *br s*	66.5 (CH)	–	2α	2α, 2β, 4
4	5.71 *s*	129.9 (CH)	2, 6, 10	1, 3	3, 6β
5	–	137.3 (C)	–	–	–
6	1.18α	27.72 (CH_2)	–	7α	7α, 7β
	2.20β		8	–	4, 7α, 7β
7	2.18α	28.8 (CH_2)	–	–	6α, 6β, 8
	1.12β			9α	6α, 6β, 8
8	1.82	43.3 (CH)	14	18, 19	7α, 7β, 9
9	1.22	49.4 (CH)	–	7α	8, 11a, 11b
10	–	53.1 (C)	–	–	–
11	1.50a	21.4 (CH_2)	–	–	9, 12α
	1.88b				9, 12α
12	1.24α	40.0 (CH_2)	–	17	11
	1.60β		13	18	11
13	–	49.2 (C)	–	–	–
14	–	83.8 (C)	–	–	–
15	2.46α	39.7 (CH_2)	–	16α	16
	1.80β		13, 14, 16, 17	18	16
16	5.47 *dt* (1.3, 8.8)	73.2 (CH)	14, 16-O*C*OCH$_3$	15α, 17	15α, 15β, 17
17	2.84 *d* (8.8)	56.8 (CH)	13, 14, 15, 16, 20, 21, 22	12α, 16α, 21	16
18	0.77 *s*	16.3 (CH_3)	12, 13, 14, 17	8, 21, 22	–
19	9.75 *s*	202.9 (CH)	10	1, 8, 2β	–
20	–	116.5 (C)	–	–	–
21	7.21 *d* (2.5)	151.0 (CH)	17, 23	17	22, 23
22	7.95 *dd* (2.5,9.8)	148.8 (CH)	17, 24	18, 23	21, 23
23	6.17 *d* (9.8)	113.2 (CH)	24	22	22
24	–	161.8 (C)	–	–	–
16-OCO*C*H$_3$	1.84 *s*	20.9 (CH_3)	16-O*C*OCH$_3$	18	–
16-O*C*OCH$_3$	–	169.9 (C)	–	–	–

[a] NMR data obtained in $CDCl_3$, 400 MHz.
[b] NMR data obtained in $CDCl_3$, 100 MHz.

Table 2
^{1}H, ^{13}C, HMBC, NOESY and COSY NMR data for compound **2** and ^{1}H, ^{13}C NMR data for compound **3**

	^{1}H NMR data for compound **2**[a]	^{13}C NMR data for compound **2**[b]	HMBC correlations H → C	NOESY correlations	COSY correlations	^{1}H NMR data for compound 3[a]	^{13}C NMR data for compound 3[b]
1	1.07α	46.9 (CH_2)	19	1β, 3, 9	2	1.28α	47.5 (CH_2)
	3.00β		2, 3, 5, 10	1α	2	2.69β	
2	4.57 *ddd* (13.6, 2.9, 7.2)	69.1 (CH)	4	2′, 19	1α, 1β, 3	4.61 *m*	70.4 (CH)
3	3.65 *dd* (3.8, 7.6)	83.1 (CH)	2	4	2, 4	3.86 *dd* (3.9, 9.7)	82.4 (CH)
4	4.28	77.9 (CH)	5, 6	3	3	5.61 *d* (3.8)	79.2 (CH)
5	–	139.8 (C)	–	–	–	–	136.6 (C)
6	5.72	124.1 (CH)	4, 10	4	7	5.91 *m*	128.9 (CH)
7	2.32	35.1 (CH)	5, 6, 8, 9	6	6	2.44	35.9 (CH)
8	–	74.4 (C)	–	–	–	–	75.5 (C)
9	1.63	51.9 (CH)	11, 19	4	11	2.09	48.6 (CH_2)
10	–	39.4 (C)	–	–	–	–	40.3 (C)
11	5.03 *d* (11.9)	71.6 (CH)	10, 12	18, 19	9	6.03 *d* (12.4)	76.2 (CH)
12	–	213.9 (C)	–	–	–	–	211.7 (C)
13	–	62.6 (C)	–	–	–	–	64.4 (C)
14	–	86.1 (C)	–	–	–	–	87.0 (C)
15	1.39	34.8 (CH_2)	13, 14, 17, 18	–	16α, 16β	1.50	30.7 (CH_2)
16	2.00α	27.8 (CH_2)	–	16α	15, 17	1.98α	29.0 (CH_2)
	1.67β			17	15, 17	1.77β	
17	4.14 *dd* (7.5, 9.3)	41.5 (CH)	–	16α, 21	16α, 16β	4.05 *m*	42.4 (CH)
18	1.11 *s*	18.8 (CH_3)	12, 13, 14, 17	11, 22	–	1.15 *s*	19.4 (CH_3)
19	1.68 *s*	19.2 (CH_3)	5, 9, 10	–	–	1.45 *s*	20.0 (CH_3)
20	–	122.1 (C)	–	–	–	–	123.1 (C)
21	7.51 *d* (2.5)	150.3 (CH)	20, 24	17	22	7.48 *d* (2.5)	151.5 (CH)
22	7.95 *dd* (2.5, 9.7)	147.9 (CH)	–	18, 23	21, 22, 23	7.92 *dd* (2.5, 9.6)	148.9 (CH)
23	6.31 *d* (9.7)	114.7 (CH)	20, 24	22	22	6.30 *d* (9.6)	115.9 (CH)
24	–	163.3 (C)	–	–	–	–	164.4 (C)
1′	5.20	99.7 (CH)	3, 2′, 3′, 5′	–	2′	5.07 *s*	98.6 (CH)
2′	4.28	65.5 (CH)	–	–	1′	5.48 *s*	67.5 (CH)
3′	–	100.2 (C)	–	–	–	–	99.7 (C)
4′	1.82α	36.9 (CH_2)	5′, 6′	1′, 4′α	5′	1.69	36.1 (CH_2)
	1.61β		–	4′β	5′		
5′	4.28 *m*	70.4 (CH)	–	–	4′, 6′	4.33 *m*	71.4 (CH)
6′	1.18 *d* (6.2)	20.7 (CH_3)	1′, 4′, 5′	2′	5′	1.20 *d* (6.0)	21.8 (CH_3)

[a] NMR data obtained in CD_3OD, 400 MHz.
[b] NMR data obtained in CD_3OD, 100 MHz.

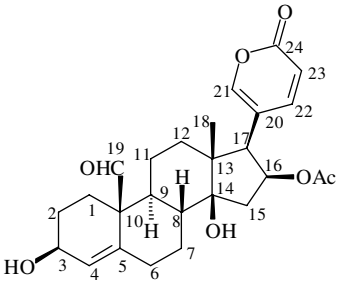
usually indicates C-14 when a 14β-hydroxyl group is present – a characteristic feature of most known bufadienolides (Dewick, 2002; Van Heerden et al., 1988). The correlation between the 3H-18 resonance and the quaternary carbon resonance at δ83.8 (C) assigned to C-14, in the HMBC spectrum, confirmed the presence of the hydroxyl group at C-14. The doublet of triplets at δ5.47 ($J = 1.3$, 8.8 Hz) in the ^{1}H NMR spectrum, ascribed to H-16 showed correlations to the C-14 resonance in the HMBC spectrum, and was also seen to be coupled to the doublet at δ2.84 ($J = 8.8$ Hz), ascribed to H-17, in the COSY spectrum. The downfield shift of the H-16 proton at δ5.47, as well as its correlation to the acetate carbonyl carbon resonance at δ169.9 in the HMBC spectrum indicated that an acetate group was present at C-16. A correlation between the H-16 resonance and the H-17 resonance in the NOESY spectrum indicated that H-16 was α-orientated.

A correlation between the resonance at δ1.82 ascribed to H-8 and the C-14 resonance was observed in the HMBC spectrum. The correlations observed between the H-8β resonance and the 3H-18 resonance in the NOESY spectrum confirmed this assignment. The H-8 resonance was seen to be coupled to the H-9 (δ1.22) and 2H-7 (δ2.18, 1.12) resonances and the 2H-7 resonances, in turn, were seen to be coupled to the 2H-6 (δ1.18, δ2.20) resonances in the COSY spectrum. The 2H-6 resonances were not further coupled indicating a 4,5-double bond with C-4 and C-5 resonances occurring at δ129.9 and δ137.3, respectively. The corresponding H-4 resonance occurred at δ5.71 and was seen to be coupled to a methine resonance at δ4.15 ascribed to H-3 in the COSY spectrum, which also indicated the presence of two other sets of methylene protons in the same spin system and they were assigned to 2H-1 and 2H-2. The corresponding C-3 resonance occurred at δ66.5 and a hydroxyl group was placed at this position. The 2H-1 resonance at δ2.34 showed correlations to the quaternary carbon resonance at δ53.1, ascribed to C-10, as well as to the resonance at δ202.9 (CH) which was assigned to the aldehyde group carbon at C-19. A correlation between the H-19 resonance (δ9.75) and the H-8β resonance at δ1.82 in the NOESY spectrum, confirmed the β-orientation of the aldehyde group. The NMR data for compound **1**

correlated well with that of other bufadienolides containing aldehydes in the same position (Van Heerden et al., 1988). Compound **1** was identified as scillicyanosidin which has been isolated before from the enzyme hydrolysis of scillicyanoside but not before as a natural product (Lichti et al., 1973).

The ^{1}H NMR spectrum of compound **2** indicated the presence of a bufadienolide δ-pyrone ring with doublets at δ7.51 ($J = 2.5$ Hz) and δ6.31 ($J = 9.7$ Hz) and a doublet of doublets at δ7.95 ($J = 2.5$, 9.7 Hz) which were assigned to H-21, H-23 and H-22, respectively. The NOESY spectrum showed correlations between the H-21 resonance and a doublet of doublets at δ4.14, ascribed to H-17. The singlet at δ1.11 was assigned to 3H-18 due to a correlation to the corresponding C-17 resonance at δ41.5 in the HMBC spectrum. Six HC–O resonances were indicated by methine resonances at δ83.1, δ77.9, δ71.6, δ70.4, δ69.1 and δ65.5 in the ^{13}C NMR spectrum and corresponding resonances at δ3.65, δ4.28, δ5.03, δ4.28, δ4.57 and δ4.28 in the ^{1}H NMR spectrum. Quaternary C–O carbon resonances at δ100.2, δ86.1 and δ74.4 were also observed. The 3H-18 resonance showed HMBC correlations to the resonance at δ86.1 (C) ascribed to C-14, and a hydroxyl group was placed at C-14 as in **1**. The TOCSY spectrum confirmed this as it showed correlations between H-17, and the 2H-16 and 2H-15 protons only. The 3H-18 resonance also showed correlations in the HMBC spectrum to a carbonyl carbon resonance at δ213.9 ascribed to C-12. Correlations were also observed between the methine proton resonance at δ5.03 ascribed to H-11 and the C-12 resonance, and a hydroxyl group was placed at C-11. The resonance ascribed to H-11 gave a correlation with the β-orientated 3H-18 (δ1.11) and 3H-19 (δ1.68) proton resonances in the NOESY spectrum indicating that H-11 was in the β-configuration. The COSY spectrum showed that the H-11 doublet at δ5.03 was only coupled to a resonance at δ1.63 ascribed to H-9 and that H-9 was not further coupled. A hydroxyl group was thus placed at C-8. The resonance at δ2.32, ascribed to 2H-7, showed correlations to the quaternary carbon resonance at δ74.4 (C), ascribed to C-8, in the HMBC spectrum, and was coupled only to the H-6 proton resonance at δ5.72 in the COSY spectrum confirming that a hydroxyl group was present at C-8. The stereochemistry at C-8 and C-14 could not be confirmed but a literature search showed that in 8,14-hydroxybufadienolides, the hydroxyl groups are always β (Pohl et al., 2000).

A double bond was indicated by resonances at δ124.1 (CH) and δ139.8 (C) in the ^{13}C NMR spectrum. The corresponding proton resonance at δ5.72 was ascribed to H-6 previously. The methine proton resonance at δ4.28, ascribed to H-4, showed correlations to C-6 as well as to the tertiary carbon resonance at δ139.8, ascribed to C-5, in the HMBC spectrum. The alkene group was placed at C-5 and a secondary hydroxyl group at C-4. The doublet of doublets ($J = 3.8$, 7.6 Hz) at δ3.65, ascribed to H-3, was seen to be coupled with H-4 as well as with the double doublet at δ4.57 ($J = 13.6$, 2.9, 7.2 Hz) ascribed to H-2 in the COSY spectrum. The HMBC spectrum showed correlations between the H-1 resonance at δ1.07 and the methyl carbon resonance at δ19.2, ascribed to C-19. The stereochemistry at C-2, C-3 and C-4 was determined from the NOESY spectrum. The H-9 resonance which was assigned as α-orientated on biosynthetic grounds, gave a positive correlation with the H-4 resonance in the NOESY spectrum, indicating that it was α-orientated, and the H-4α resonance, in turn, showed a correlation with the H-3 resonance indicating that it was α-orientated. The resonance which was assigned to H-2 showed a positive NOESY correlation with the 3H-19 resonance indicating that H-2 was β. The stereochemistry of H-2β and H-3α was confirmed by the coupling constant of ≈7 Hz (Steyn et al., 1986).



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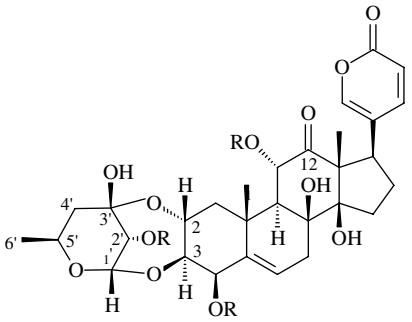

2 R=H; **3** R=OAc

Two resonances which appeared to be due to hemiacetal and hemiketal carbons were observed at δ99.7 (CH) and δ100.2 (C), respectively in the ^{13}C NMR spectrum. These resonances, together with the four remaining unassigned carbon resonances at δ65.5 (CH), δ36.9 (CH_2), δ70.4 (CH) and δ20.7 (CH_3) could be ascribed to a sugar. Correlations between the hemiacetal proton resonance at δ5.20 ascribed to H-1′ and C-3 in the HMBC spectrum as well as positive correlations in the NOESY spectrum between H-2 and a resonance at δ4.28 which forms part of the sugar moiety indicated that the sugar was attached to both C-2 and C-3. Overlapping of resonances at δ4.28 made it difficult to determine the nature of the sugar and compound **2** was thus acetylated to form the 4,11,2′-triacetate, **3**. The resonances H-4 (δ4.28), H-11 (δ5.03) and H-2′ (δ4.28)

showed downfield shifts to δ5.61, δ6.03 and δ5.48, respectively in the ^{1}H NMR spectrum of compound **3** (Table 2). Correlations seen in the COSY spectrum between the resonances of H-1′ (hemiacetal carbon at δ98.6) and H-2′, H-5′ and 2H-4′ as well as H-5′ and 3H-6′ enabled the assignment of all the glycosidic resonances. The COSY spectrum also made it possible to differentiate between two spin systems in the sugar moiety containing the H-1′ and H-2′ protons and the 2H-4′, H-5′ and 3H-6′ protons, respectively. Correlations were observed in the NOESY spectrum between the H-2′β proton resonance and the resonances of H-1′β and H-2β. The H-1′ and H-2′ resonances showed correlations to the hemiketal carbon resonance at δ99.7 (C), ascribed to C-3′, in the HMBC spectrum. Because of the necessity of a *cis*-fusion in bridged six-membered rings, the hydroxyl group at C-3′ should adopt the β-orientation. Finally NOESY correlations between the proton resonance ascribed to H-5′α at δ4.33 and H-3α allowed the assignment of the β-orientation to the methyl group in position 6′. The sugar moiety was identified as a 2′-epimer of 4′-deoxyrhamnose, or 4,6-dideoxy-L-glucose.

Compound **2** was identified as a novel bufadienolide, lydenburgenin which is related to rubellin, identified as the main toxic compound of *Drimia modesta* (Baker) Jessop (syn. *Urginea rubella* Baker) (Steyn et al., 1986). The current findings are of chemotaxonomic interest: when *Drimia delagoensis* (reportedly conspecific with *U. lydenburgensis*) (Jessop, 1977) was phytochemically characterised, no bufadienolides could be found, but rather a novel homoisoflavanoid and a simple aromatic acid (Koorbanally et al., 2005). Such a difference in chemical profiles supports the recognition of two distinct taxa.

3. Experimental

Fresh bulbs of *U. lydenburgensis* R.A. Dyer were collected in Nelspruit, Mpumalanga, and a voucher specimen retained (*Crouch 864*, NH). The bulbs (3 kg) were macerated and extracted at room temperature with continuous agitation for 3 days with dichloromethane. The crude methylene chloride extract was subjected to column chromatography over silica gel (Merck 9385) using a step-gradient solvent system with ethyl acetate/hexane (2:3) as starting mobile phase and then increasing ratios of ethyl acetate was introduced. Five fractions were collected. Fraction 5 (ethyl acetate/hexane, 4:1) was purified by column chromatography starting with 100% ethyl acetate as mobile phase introducing a methanol gradient. Two bufadienolides (**1** and **2**) were isolated. Compound **1** (6.1 mg, 0.02%) eluted with ethyl acetate/methanol (95:5) and compound **2** (6.7 mg, 0.02%) with ethyl acetate/methanol (8:2). Compound **2** was further purified employing a chromatotron and ethyl acetate/dichloromethane/methanol (5:4.5:0.5) as mobile phase.

Compound **2** (6.7 mg) was acetylated with pyridine (1 ml) and acetic anhydride (1 ml). Water was added to the crude acetate and the mixture extracted with ethyl acetate (4×10 ml). The ethyl acetate extract was dried and yielded the acetylated product, **3** (4 mg).

NMR spectra were recorded on a Varian Inova 400 MHz NMR spectrometer. IR spectra were recorded on a Shimadzu FTIR-4300 spectrophotometer and the mass spectra on a VG (now Waters) 70-SE magnetic sector mass spectrometer by direct insertion probe using an accelerating voltage of 8 kV and a mass range of 3000 at Kent Mass Spectrometry in London. NMR data for **1**, **2** and **3** are given in Tables 1 and 2.

Scillicyanosidin (**1**): white crystalline powder (6.1 mg); m.p. 150 °C; $[\alpha]_D = +18.6°$ (*c*, 0.043 g/100 ml, $CHCl_3$); positive-ion FAB-MS: *m*/*z* (rel. int.) 457 $[M+H]^+$. EIMS: *m*/*z* (rel. int.): 456 $[M]^+$ (30), 396 $[M^+-CH_3COOH]$ (25), 378 $[M^+-CH_3COOH-H_2O]$ (8), 368 (7), 350 (12), 307 (23), 91 (100); IR: λ_{max} [KBr](cm^{-1}) 3456 (O–H stretch, 2934, 2857 (C–H stretch), 1714 (C=O stretch), 1533 (aromatic C=C stretch), 1374, 1245 (CH_2 and CH_3 bend), 1086, 1039 (C–O stretch).

Lydenburgenin (**2**): white crystals (6.7 mg); m.p. 217–219 °C $[\alpha]_D = +47.6°$ (*c*, 0.042 g/100 ml, $CHCl_3$). MS not acquired.

Lydenburgenin acetate (2″,4,11-triacetoxylydenburgenin) (**3**): colourless oil (4.0 mg); positive-ion FAB-MS: *m*/*z* (rel. int.): 731 $[M+15]^+$; EIMS: *m*/*z* 429 $[M^+$–glycoside–$2 \times CH_3COOH]$ (1), 149 (28), 84 (97), 49 (100).

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