

## Eucosterol-Type Nortriterpenoids from *Merwillia natalensis*

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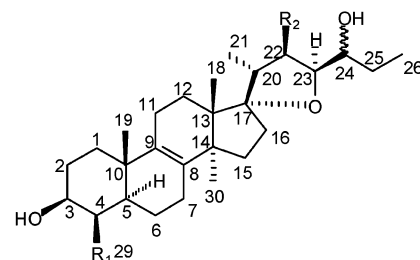
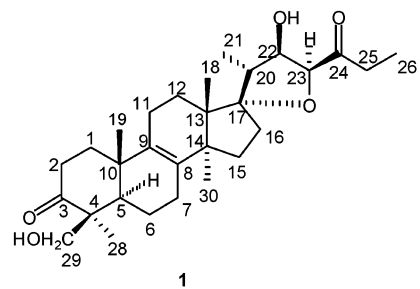
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The bulbs of *Merwillia natalensis* have yielded two known homoisoflavanones, the known spirocyclic homoisoflavanone, scillascillin, four known nortriterpenoids, and the new nortriterpenoid, (22*R*,23*S*)-17 $\alpha$ ,23-epoxy-22,29-dihydroxy-27-nor-lanost-8-en-3,24-dione (**1**), bisnortriterpenoid, (22*R*,23*S*)-17 $\alpha$ ,23-epoxy-3 $\beta$ ,22,24 $\xi$ -trihydroxy-27,28-bisnor-lanost-8-ene (**2**), and trisnortriterpenoid, (23*S*)-17 $\alpha$ ,23-epoxy-3 $\beta$ ,24 $\xi$ -dihydroxy-27,28,29-trisnor-lanost-8-ene (**3**). The structures of **1–3** were determined by spectroscopic methods.

*Merwillia natalensis* (Planch.) Speta (syn. *Scilla natalensis* Planch.) (Hyacinthaceae) is used by various ethnic groups in southern Africa to treat a range of ailments. The bulbs are the most popular item traded in the ethnomedical plant markets of Durban,<sup>1</sup> and it is becoming increasingly rarer; the species was recently Red Data Listed as "Vulnerable".<sup>2</sup> The Zulu use bulb decoctions as an ingredient in infusions taken to facilitate labor at birth.<sup>3</sup> The Southern Sotho eat the cooked bulbs with food as an aperient, treat "internal tumors" with bulb decoctions, and rub powdered bulbs into scarifications over sprains and fractions.<sup>4,5</sup> The Tswana rub the powdered bulbs into the back joints and other parts in the belief that it increases their strength and resilience to witchcraft.<sup>5</sup> In the treatment of veld sores and boils, the Swati apply a lotion prepared by boiling the macerated bulbs in water.<sup>5</sup>

A previous investigation of two collections of this species (reported as *Scilla natalensis*), sourced from the Kwazulu-Natal and Mpumalanga Provinces in South Africa, yielded two homoisoflavanones, 5,7-dihydroxy-6-methoxy-3-(4-hydroxybenzyl)chroman-4-one and 5,7-dihydroxy-6-methoxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one, but no eucosterol-type compounds.<sup>1</sup> The current report details analysis of a further collection of *M. natalensis* found growing half submerged in a swamp, which has yielded the same homoisoflavanones earlier reported,<sup>1</sup> and the spirocyclic homoisoflavanone scillascillin, which has been isolated previously from *Scilla scilloides* (Lindl.) Druce<sup>6</sup> and *Muscari neglectum* Guss. ex Ten.<sup>7</sup> An additional seven eucosterol-type compounds were identified: (22*R*,23*S*)-17 $\alpha$ ,23-epoxy-3 $\beta$ ,22,29-trihydroxy-27-nor-lanost-8-en-24-one, (23*S*)-17 $\alpha$ ,23-epoxy-3 $\beta$ ,28,29-trihydroxy-27-nor-lanost-8-en-24-one, (22*R*,23*S*)-22-acetoxy-17 $\alpha$ ,23-epoxy-3 $\beta$ ,29-dihydroxy-27-nor-lanost-8-en-24-one, (23*S*)-17 $\alpha$ ,23-epoxy-3 $\beta$ ,29-dihydroxy-27-nor-lanost-8-en-24-one, (22*R*,23*S*)-17 $\alpha$ ,23-epoxy-22,29-dihydroxy-27-nor-lanost-8-en-24-one, (22*R*,23*S*)-17 $\alpha$ ,23-epoxy-22,29-dihydroxy-27-nor-lanost-8-en-3,24-dione (**1**), (22*R*,23*S*)-17 $\alpha$ ,23-epoxy-3 $\beta$ ,22,24 $\xi$ -trihydroxy-27,28-bisnor-lanost-8-ene (**2**), (23*S*)-17 $\alpha$ ,23-epoxy-3 $\beta$ ,24 $\xi$ -dihydroxy-27,28,29-trisnor-lanost-8-ene (**3**). Three of these, compounds **1–3**, have not been reported previously and are the subject of this paper.

The four known 27-nor-triterpenoids isolated possess a 8,9-double bond and a  $\beta$ -substituted hydroxy group at C-3



	R <sub>1</sub>	R <sub>2</sub>
<b>2</b>	CH <sub>3</sub>	OH
<b>3</b>	H	H

and have the C-29 methyl group oxidized to a primary alcohol (to give a pair of H-29 doublets in the <sup>1</sup>H NMR spectrum), a keto group at C-24, and a fifth ring formed by an ether linkage between C-17 and C-23. The compounds differ from each other in the substitution at C-22 $\beta$  (unsubstituted, hydroxy, or acetoxy) and at C-28, which has been oxidized to a second primary alcohol in (23*S*)-17 $\alpha$ ,23-epoxy-3 $\beta$ ,28,29-trihydroxy-27-nor-lanost-8-en-24-one.

Compound **1** was isolated as a white crystalline material, and the NMR spectra showed it to be similar in structure to the four known eucosterol-type nor-triterpenoids also obtained in the present investigation. The HRMS indicated a molecular formula of C<sub>29</sub>H<sub>44</sub>O<sub>5</sub>. The IR spectrum showed hydroxyl and carbonyl stretch bands at 3439 and 1701 cm<sup>-1</sup>, respectively. The structure of compound **1** differed from that of (22*R*,23*S*)-17 $\alpha$ ,23-epoxy-3 $\beta$ ,22,29-trihydroxy-27-nor-lanost-8-en-24-one in that a keto group was present at C-3 instead of a hydroxyl group. This placement of the keto group was indicated by HMBC correlations between the C-3 resonance ( $\delta$  214.5) and the two H-29 proton doublets at  $\delta$  3.63 and 4.24. The C-8, C-9 double bond was indicated by resonances at  $\delta$  135.7 and 133.6, while C-17 occurred in a typically downfield position of  $\delta$  96.7, which,

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**Table 1.** NMR Data for Compound **1** (C<sub>5</sub>D<sub>5</sub>N+ DMSO), Compound **2** (C<sub>5</sub>D<sub>5</sub>N), and Compound **3** (CDCl<sub>3</sub>) (*J* values in Hz)

	<b>1</b>		<b>2</b>		<b>3</b>	
	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$	$\delta_{\text{H}}$	$\delta_{\text{C}}$
1 $\alpha$	1.93 (m) (2H)	20.7	1.05 (m)	35.5	1.92 (m) (2H)	21.4
1 $\beta$			1.65 (m)			
2 $\alpha$	1.32 (m) (2H)	36.6	1.72 (m)	32.6	1.30 (m)	35.0
2 $\beta$			1.93 (m)		1.62 (m)	
3		214.5	3.16 (m)	75.6	3.60 (m)	71.2
4		54.4	1.41 (m)	39.9	1.15 (m)	38.3
					1.72 (m)	
5	1.50	52.9	0.89	47.5	1.32 (m)	40.4
6 $\alpha$	1.63 (m)	19.1	1.93 (m)	21.8	1.96 (m)	41.0
6 $\beta$	1.53 (m)		2.06 (m)		1.75 (m)	
7 $\alpha$	1.98 (m) (2H)	26.0	1.16 (m)	21.2	2.06 (m)	21.4
7 $\beta$			1.67 (m)			
8		135.7		135.7		135.2
9		133.6		134.1		133.8
10		36.8		36.6		35.6
11 $\alpha$	2.78 (m)	35.2	2.06 (m)	25.7	1.82 (m)	31.6
11 $\beta$	2.21 (m)		1.92 (m)		1.40 (m)	
12 $\alpha$	1.36 (m)	24.8	1.42 (m) (2H)	25.6	2.08 (m)	24.8
12 $\beta$	2.30 (m)				2.20 (m)	
13		49.6		50.3		50.6
14		49.3		50.1		48.7
15 $\alpha$	1.34 (m)	32.0	1.30 (m)	32.2	1.32 (d, 1.31)	31.7
15 $\beta$	1.59 (m)		1.55 (m)		1.54 (d, 1.65)	
16 $\alpha$	2.21 (m)	33.0	2.68 (m)	41.2	1.15 (m)	35.0
16 $\beta$	2.23 (m)		2.28 (m)		1.72 (m)	
17		96.7		95.3		95.6
18	0.84 (s)	18.9	0.88 (s)	19.6	0.91 (s)	19.1
19	1.13 (s)	19.2	0.90 (s)	18.4	0.92 (s)	17.1
20	2.35 (m)	51.6	2.39 (m)	53.2	2.18 (m)	44.7
21	0.94 (d, 7.3)	15.6	0.99 (d, 7.1)	16.5	1.04 (d, 6.8)	17.6
22	4.42 (bs)	79.3	4.13 <sup>a</sup>	81.2	1.51 (dd, 6.0, 6.2)	36.4
					1.66 <sup>a</sup>	
23	4.74 (d, 5.1)	87.2	4.01 <sup>a</sup>	83.0	3.82 (m)	80.9
24		210.4	4.07 (m)	73.6	3.35 (m)	78.9
25	2.51 (q, 7.3)	33.0	1.75 (m)	27.9	1.40 (m)	25.6
	2.61 (q, 7.5)					
26	0.96 <sup>a</sup>	7.1	1.05 (t, 7.3)	10.9	0.99 (t, 7.3)	9.9
28	1.27 (s)	19.9				
29	3.63 (d, 11.0)	64.4	1.13 (d, 6.2)	15.8		
	4.24 (d, 11.0)					
H-30	1.40 (s)	26.0	1.33 (s)	26.9	1.11 (s)	26.5

<sup>a</sup> Peaks obscured, *J* could not be determined.

with the molecular formula, confirmed the C-17, C-23 ether linkage as in the known analogues also isolated. The methyl group proton singlet resonances at  $\delta$  0.84, 1.13, 1.27, and 1.40 could be assigned to H<sub>3</sub>-18, -19, -28, and -30, on the basis of HMBC and NOESY correlations. The structure of the side chain was confirmed in the following manner: the H<sub>3</sub>-21 methyl group proton doublet at  $\delta$  0.94 was seen to be coupled to the H-20 multiplet at  $\delta$  2.35 in the COSY spectrum. This was, in turn, coupled to a methine proton resonance at  $\delta$  4.42 (H-22), which was further coupled to the H-23 methine resonance at  $\delta$  4.74. The H-23 resonance was not further coupled. An isolated terminal ethyl group was indicated by typical quartet and triplet resonances at  $\delta$  2.51 (2H-25) and 0.96 (H<sub>3</sub>-26). The C-24 keto group carbon resonance occurred at  $\delta$  210.4. The stereochemistry of the eucosterol-type side chain was confirmed by NOESY correlations between H<sub>3</sub>-21, H-22 and H-23, as expected. Thus, the structure of **1** was determined to be (2*R*,23*S*)-17 $\alpha$ ,23-epoxy-22,29-dihydroxy-27-nor-lanost-8-en-3,24-dione.

The HRMS of **2** gave a molecular formula of C<sub>28</sub>H<sub>46</sub>O<sub>4</sub>, indicating a bisnor-triterpenoid. The IR spectrum showed a hydroxyl stretch band at 3393 cm<sup>-1</sup> but no carbonyl stretch band. The primary alcohol present at C-29 in **1** was absent, and the presence of a methyl group proton doublet was seen at  $\delta$  1.13. The corresponding <sup>13</sup>C NMR resonance at  $\delta$  15.8 showed HMBC correlations with H-3 ( $\delta$  75.6) and

H-5 ( $\delta$  47.5) and a resonance at  $\delta$  1.41. The COSY spectrum showed coupling between H-3, H-5, and the resonance at  $\delta$  1.41 which was assigned to H-4. Thus, it appeared that a carbon atom attached to C-4 had been lost compared to **1**. The NOESY spectrum showed correlations between H-5 $\alpha$  and H-4 and between H-19 (which is  $\beta$ ) and the methyl group proton doublet. Accordingly, the methyl group at C-4 was assigned with  $\beta$  stereochemistry.

The keto group present at C-24 in the other eucosterol-type compounds isolated was not present in compound **2**, which had a hydroxy group present at this position. This was shown in the COSY spectrum, where coupling between H<sub>3</sub>-21 ( $\delta$  0.99), H-20 ( $\delta$  2.39), H-22 ( $\delta$  4.13), H-23 ( $\delta$  4.01), H-24 ( $\delta$  4.07), H<sub>2</sub>-25 ( $\delta$  1.75), and H<sub>3</sub>-26 ( $\delta$  1.05) could be seen. Thus **2** was assigned the structure (2*R*,23*S*)-17 $\alpha$ ,23-epoxy-3 $\beta$ ,22,24 $\xi$ -trihydroxy-27,28-bisnor-lanost-8-ene. The stereochemistry at C-24 could not be determined. The synthesis of Mosher esters was attempted but was unsuccessful due to the compound decomposing on reaction.

The HRMS of compound **3** indicated a molecular formula of C<sub>27</sub>H<sub>44</sub>O<sub>3</sub>, indicating the loss of a further carbon atom. As in the IR spectrum of compound **2**, a hydroxyl group stretch band was present, but no carbonyl stretch band. Two additional methylene carbons were seen to be present in the <sup>13</sup>C NMR spectrum replacing methyl and oxymethine carbons. One of these methylene carbons ( $\delta$  36.4) was assigned to C-22, and the corresponding proton resonances

occurred at  $\delta$  1.51 and 1.66. This was indicated by the COSY spectrum, which showed a chain of coupled resonances from H<sub>3</sub>-21 ( $\delta$  1.04), to H-20 ( $\delta$  2.18), to H<sub>2</sub>-22 ( $\delta$  1.51, 1.66), to H-23 ( $\delta$  3.82), to H-24 ( $\delta$  3.35), to H<sub>2</sub>-25 ( $\delta$  1.40), to H<sub>3</sub>-26 ( $\delta$  0.99). This again confirmed the presence of a hydroxy group at C-24 rather than the usual eucosterol-type keto group.

The <sup>1</sup>H NMR spectrum showed the presence of only five methyl group proton resonances in **3**. The presence of methyl groups at C-21 and C-26 was already established from the COSY spectrum. The remaining three methyl group proton resonances all occurred as singlets and using the HMBC spectrum could be assigned to H<sub>3</sub>-18 ( $\delta$  0.91), H<sub>3</sub>-19 ( $\delta$  0.92), and H<sub>3</sub>-30 ( $\delta$  1.11). This suggested that a further methyl group had been lost from C-4. This was confirmed by COSY correlations between H-3 ( $\delta$  3.60), H<sub>2</sub>-4 ( $\delta$  1.15 and 1.72), and H-5 ( $\delta$  1.32). Thus the structure of **3** was assigned as (23*S*)-17 $\alpha$ ,23-epoxy-3 $\beta$ ,24 $\xi$ -dihydroxy-27,28,29-trisnor-lanost-8-ene.

### Experimental Section

**General Experimental Procedures.** Optical rotations were measured at room temperature in chloroform using either a Optical Activity AA-5 polarimeter together with a series A2 stainless steel (4 × 200 mm) unjacketed flow tube or a Perkin-Elmer 241 polarimeter with a 10 cm flow tube. IR spectra were recorded with a Nicolet Impact 400 D spectrometer on sodium chloride plates and calibrated against an air background. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on a Varian Unity INOVA 400 MHz NMR spectrometer. HRMS were obtained using a Kratos high-resolution MS 9/50 spectrometer at the Cape Technikon.

**Plant Material.** Bulbs of *Merwillia natalensis* (Planch.) Speta (Hyacinthaceae) were collected in February 2001, from the Blyde Nature Reserve in Mpumalanga, South Africa, identified by N.R.C., and a voucher specimen retained (*N. Crouch 855*, NH).

**Extraction and Isolation.** The chopped bulbs (3.08 kg) were extracted with dichloromethane using a shaker at room temperature for 48 h. The dichloromethane extract (10.65 g) yielded three homoisoflavanones, 5,7-dihydroxy-6-methoxy-3-(4'-hydroxybenzyl)-4-chromanone<sup>8</sup> (12 mg), 5,7-dihydroxy-6-methoxy-3-(3'-hydroxy-4'-methoxybenzyl)-4-chromanone<sup>8</sup> (10 mg), and scillascillin<sup>6,7</sup> (17 mg), five nortriterpenoids, (22*R*,23*S*)-17 $\alpha$ ,23-epoxy-3 $\beta$ ,22,29-trihydroxy-27-nor-lanost-8-en-24-one<sup>9</sup> (20 mg), (23*S*)-17 $\alpha$ ,23-epoxy-3 $\beta$ ,28,29-trihydroxy-27-nor-lanost-8-en-24-one<sup>9</sup> (12 mg), (22*R*,23*S*)-22-acetoxy-17 $\alpha$ ,23-epoxy-3 $\beta$ ,29-dihydroxy-27-nor-lanost-8-en-24-one,<sup>11</sup> (15 mg), (23*S*)-17 $\alpha$ ,23-epoxy-3 $\beta$ ,29-dihydroxy-27-nor-lanost-8-en-24-one<sup>9</sup> (15 mg), and (22*R*,23*S*)-17 $\alpha$ ,23-epoxy-22,29-dihydroxy-27-nor-lanost-8-en-3,24-dione (**1**, 30 mg), a bisnortriterpenoid, (22*R*,23*S*)-17 $\alpha$ ,23-epoxy-3 $\beta$ ,22,24 $\xi$ -trihydroxy-27,28-bisnor-lanost-8-ene (**2**, 30 mg), and a trisnortriterpenoid, (23*S*)-17 $\alpha$ ,

23-epoxy-3 $\beta$ ,24 $\xi$ -dihydroxy-27,28,29-trisnor-lanost-8-ene (**3**, 20 mg), after column chromatography over silica gel (Merck 9385). The known compounds were identified using NMR and MS techniques and were confirmed by comparison against literature values.

**(22*R*,23*S*)-17 $\alpha$ ,23-Epoxy-22,29-dihydroxy-27-norlanost-8-en-3,24-dione (**1**):** white crystals (MeOH); mp 88–91 °C; [ $\alpha$ ]<sub>D</sub> –19° (*c* 0.084, CHCl<sub>3</sub>); IR (KBr)  $\nu_{\max}$  3439, 2949, 1701 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, see Table 1; HREIMS *m/z* 472.31953 (calcd for C<sub>29</sub>H<sub>44</sub>O<sub>5</sub>, 472.318875).

**(22*R*,23*S*)-17 $\alpha$ ,23-Epoxy-3 $\beta$ ,22,24 $\xi$ -trihydroxy-27,28-bisnor-lanost-8-ene (**2**):** yellowish white gum; [ $\alpha$ ]<sub>D</sub> +8.1° (*c* 0.080, CHCl<sub>3</sub>); IR (NaCl)  $\nu_{\max}$  3393, 2924, 2853 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, see Table 1; HREIMS *m/z* 446.33844 (calcd for C<sub>28</sub>H<sub>46</sub>O<sub>5</sub>, 446.339610).

**(23*S*)-17 $\alpha$ ,23-Epoxy-3 $\beta$ ,24 $\xi$ -dihydroxy-27,28,29-trisnor-lanost-8-ene (**3**):** yellow gum; [ $\alpha$ ]<sub>D</sub> +20.4° (*c* 0.054, CHCl<sub>3</sub>); IR (NaCl)  $\nu_{\max}$  3396, 2928, 2864 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, see Table 1; HREIMS *m/z* 416.32916 (calcd for C<sub>27</sub>H<sub>44</sub>O<sub>3</sub>, 416.329046).

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### References and Notes

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