

Cite this: *RSC Adv.*, 2017, 7, 15416

Received 22nd December 2016

Accepted 24th February 2017

DOI: 10.1039/c6ra28584h

rsc.li/rsc-advances

Novel triterpenoid derivatives from *Eucomis bicolor* Bak. (Hyacinthaceae: Hyacinthoideae)[†]

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The bulbs of *Eucomis bicolor* (Hyacinthoideae) yielded fourteen novel natural compounds, including (17S)-3-oxo-24,25,26,27,28-pentanorlanost-8-en-23,17 α -olide, whose structure was determined using the logic for structure determination program, and nine novel lanosterol glycosides. Compounds were screened against the NCI-59 cancer cell panel but showed limited activity.

Introduction

The genus *Eucomis* L'Hér. (Hyacinthaceae *sensu* APGII: Hyacinthoideae) has long been one of the most highly regarded sources of ethnomedicines in southern Africa, a notion based largely on its popularity in informal trade.^{1–3} *Eucomis* is a small genus of bulbous plants (ten species and fourteen currently accepted taxa), producing inflorescences that are characteristically topped by a conspicuous coma of leafy sterile bracts.³ All genus members occur in South Africa, with only two extending to south tropical Africa. The Hyacinthoideae are known to exhibit significant infraspecific chemical variation, depending on the geographical location and season of collection.^{4–6} This prompted the investigation of naturally-sourced *Eucomis bicolor* Bak. Previous investigations of *E. bicolor*, purchased from commercial sources in the Netherlands^{7,8} and Japan,⁹ yielded, 5-hydroxy-7-methoxy-3-(4'-methoxybenzyl)-4-chromanone, 3,5-dihydroxy-7-methoxy-3-(4'-methoxybenzyl)-4-chromanone,⁸ (Z)-5,7-dihydroxy-3-(4'-methoxybenzylidene)-4-chromanone,⁷ six eucosterol oligosaccharide derivatives⁹ and scillasponin A.¹⁰

Results and discussion

The current report details the analysis of a collection of *Eucomis bicolor* Bak. sourced from the foothills of the Drakensberg mountains, KwaZulu-Natal, South Africa. The dichloromethane

extract of *Eucomis bicolor* yielded thirteen compounds, including three known homoisoflavonoids, 3,5,7-trihydroxy-3-(4'-methoxybenzyl)-4-chromanone previously isolated from *E. bicolor*,⁷ *Merwillia dracomontana* (Hilliard & B. L. Burtt) Speta (as *Scilla dracomontana* Hilliard & B. L. Burtt),¹¹ *Ledebouria graminifolia* (Bak.) Jessop,¹² *Eucomis montana* Compton and *Resnova humifusa* (Baker) U. & D. M. -D.,¹³ (R)-5,7-dihydroxy-8-methoxy-3-(4'-methoxybenzyl)-4-chromanone previously isolated from *Eucomis autumnalis* (Mill.) Chitt. subsp. *autumnalis* and *Eucomis comosa* (Houtt.) Wehrh. var. *comosa*¹⁴ and (R)-5,7-dihydroxy-3-(4'-methoxybenzyl)-4-chromanone previously isolated from *E. bicolor*,⁸ *E. montana*, *R. humifusa*,¹³ and *Ledebouria revoluta* (L.f.) Jessop,¹⁵ four known nortriterpenoids, eucosterol previously isolated from *E. autumnalis*,¹⁶ *Muscari comosum* Mill.,¹⁷ *Eucomis pallidiflora* Bak. subsp. *pole-evansii* (N.E.Br.) Reyneke ex J.C. Manning and *Eucomis schiiffii* Reyneke,³ 3-dehydro-15-deoxoeucosterol and 15-deoxoeucosterol, previously isolated from *Scilla scilloides* Druce,¹⁸ *M. comosum*,¹⁷ *Merwillia plumbea* (Lindl.) Speta (as *M. natalensis* (Planch.) Speta⁴ and *Pseudoprospero firmifolium* (Baker) Speta,¹⁹ (23S)-17 α ,23-epoxy-3 β ,28,29-trihydroxy-27-norlanost-8-en-24-one, previously isolated from *M. comosum*,²⁰ *M. plumbea*,⁴ *E. schiiffii*,³ and *Ledebouria zebrina* (Bak.) S. Venter (as *Scilla zebrina* Bak.)⁵ and 2,4-dimethoxybenzaldehyde, which has not been isolated previously from this family, and five novel triterpenoid derivatives, compounds 1–5 and a range of their glycoside derivatives which were isolated from a complex mixture after acetylation, followed by separation (6Ac–14Ac). The structures of the unacetylated forms of these compounds, as they occur in the plant, are given in Fig. 1 and structures of all compounds isolated are provided in the ESI, S.1.[†]

The HREIMS of compound 1 showed a [M + Na]⁺ peak at *m/z* 407.2570 indicating a molecular formula of C₂₅H₃₆O₃ and the presence of a pentanortriterpenoid. The IR spectrum showed peaks for lactone and keto group carbonyl stretches respectively at 1767 and 1708 cm^{−1}. The ¹H, ¹³C, ¹H–¹H COSY, ¹H–¹³C HSQC, and ¹H–¹³C HMBC spectral data were used as input for

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[†] Electronic supplementary information (ESI) available. See DOI: 10.1039/c6ra28584h



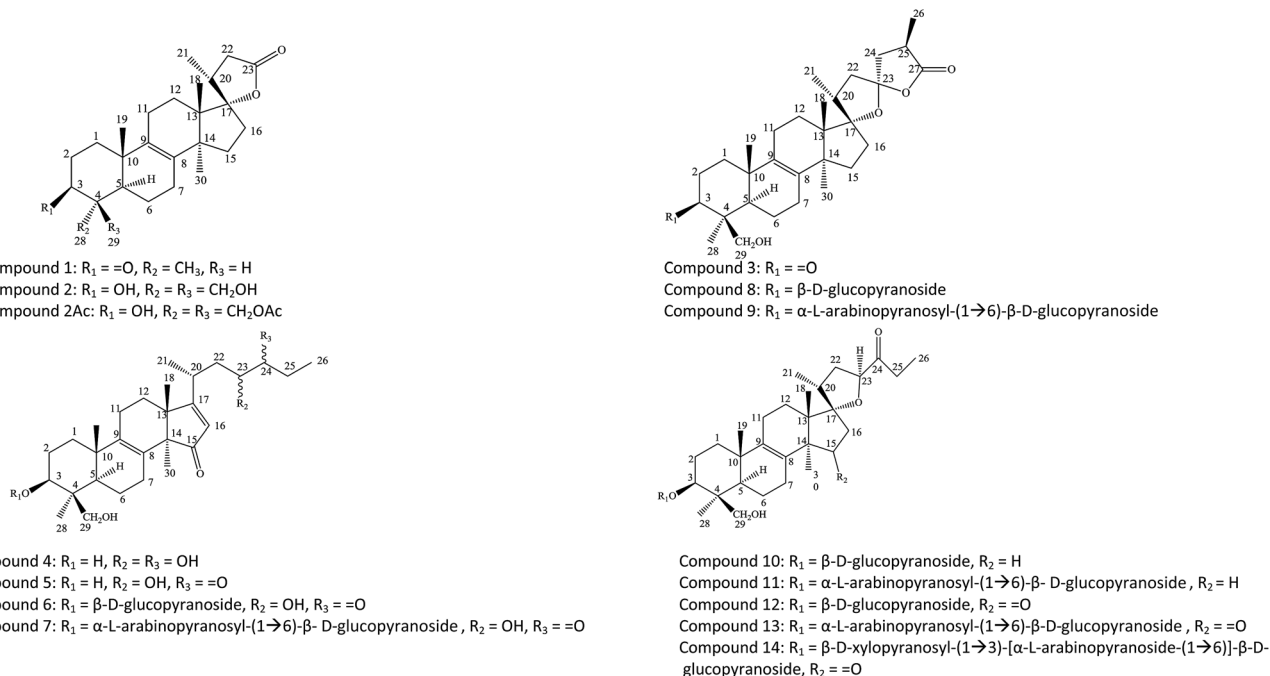


Fig. 1 Compounds isolated from *Eucomis bicolor*.

the automatic structure elucidation software LSD.^{21,22} The corresponding data file is available as ESI (S.3†). The carbon atoms were numbered in the decreasing order of their ¹³C NMR chemical shift and the hydrogen atoms were numbered so that a carbon and a hydrogen atom that are bound together have their corresponding resonances bearing the same number, according to the HSQC spectrum. The oxygen atom of the keto group was numbered 26, those from the lactone group were given the numbers 27 (sp²) and 28 (sp³). The assignment of the number of attached hydrogen atoms attached to each carbon atom by means of the *J*-modulated HSQC spectrum led to an overall number of carbonated hydrogens of 36, thus leaving no possibility for the presence of a hydroxy group. Carbons resonances at 213.1, 177.1, 135.8, and 133.1 we produced by sp² hybridized carbon atoms, while the one at 98.4 must be sp³ and bound to O-28. The HSQC and HMBC spectra were recorded with a resolution in the ¹³C domain that was sufficient to avoid any ambiguity on peak positions in this domain. However, peak overlapping in the ¹H domain led to the existence of several HMBC correlations for which the interpretation was ambiguous and considered as such by the LSD algorithm. The COSY spectrum identified the two methyl–methine bonds of the molecule and four other carbon–carbon bonds.

The carbons of the singlet methyl groups were also forced to be bonded to quaternary carbons. These constraints and the correlation data produced a single planar structure solution when processed by the LSD software. The NOESY spectrum showed correlations between the 3H-18 and 3H-19, H-20 and 3H-21 resonances, between the 3H-19/H-4 resonances and between the 3H-28/H-5 and 3H-30/H-5 resonances and between the 3H-21/H-12 α resonance. Confirming that the C-21 methyl group was in the α orientation. Thus compound 1 was

identified as (17*S*)-24,25,26,27,29-pentanor-3-oxo-lanost-8-en-23,17 α -olide. This compound would arise from a Michael-type addition of the oxygen of a C-23 hydroxy group oxygen to C-17 of a ring D α,β -unsaturated system (as seen in 4) followed by the oxidative cleavage of the lanostane C-23/C-24 bond, to yield the pentanortriterpenoid structure.

Compound 2 was isolated in very small amounts, but in larger amounts as its tri-acetyl derivative, 2Ac, after acetylation of a complex mixture. The acetate was identified as (17*S*)-24,25,26,27-tetranor-3 β ,28,29-triacetoxy-lanost-8-en-23,17 α -olide, 2Ac, hence compound 2 was the novel (17*S*)-24,25,26,27-tetranor-3 β ,28,29-trihydroxy-lanost-8-en-23,17 α -olide. The HREIMS of compound 2Ac, showed a $[\text{M} + \text{Na}]^+$ peak at m/z 581.3096 for C₃₂H₄₆O₈Na, which indicated a molecular formula of C₂₆H₄₀O₅ for 2.

Compound 2Ac differed from 1 in the structure of ring A. The ¹H NMR spectrum of 2Ac showed that the 3-keto group had been replaced by an acetoxy group with the H-3 α resonance occurring at δ 4.89 (dd, *J* = 11.60, 4.10). The corresponding C-3 resonance (δ 74.0) showed correlations in the HMBC spectrum with two sets of oxymethylene proton resonances (2H-28, δ 4.04, 4.27 ea d, *J* = 12.00; 2H-29, δ 4.31, 4.40 ea d, *J* = 12.20). The ¹³C NMR spectrum displayed three acetyl carbonyl carbon resonances at δ_{C} 170.4, δ_{C} 170.9 and δ_{C} 171.2 and HMBC correlations enabled confirmation that acetylation had taken place at C-3, C-28 and C-29.

The NOESY spectrum showed similar correlations as in 1, and showed additional correlations between the H-5/H-3 α /2H-28 resonances, confirming the configuration of the substituent at C-3 as β .

The HREIMS of 3 showed a $[\text{M} + \text{Na}]^+$ ion at m/z 507.3078 corresponding to the molecular formula C₃₀H₄₄O₅ for the



compound. The FTIR spectrum showed absorption bands at 1772 and 1701 cm^{-1} consistent with a γ -lactone and keto group respectively.²³ The keto group occurred at C-3 (δ_{C} 221.0) and the HMBC spectrum showed correlations between both the C-3 and C-5 (δ_{C} 52.1) resonances and two H-29 oxymethylene (δ_{H} 3.24, d, $J = 11.20$ Hz; δ_{H} 4.02, d, $J = 11.20$ Hz), and the 3H-28 (δ_{H} 1.28) proton resonances. The eight carbon sidechain was modified into a spiro- γ -lactone as in compounds previously reported from *Chionodoxa luciliae* Boiss.²³ The C-17 resonance (δ_{C} 98.7) was seen to correlated with the 3H-18 (δ_{H} , H-20 (δ_{H} 2.28), 3H-21 (d, δ_{H} 1.05) and two H-22 (δ_{H} 1.78 and δ_{H} 2.72) resonances in the

HMBC spectrum, and the C-23 (δ_{C} 113.4) resonance showed correlations seen with the H-20, two H-22, two H-24 (δ_{H} 2.00, δ_{H} 2.47) and H-25 (δ_{H} 2.97) resonances. The C-27 lactone carbonyl resonance (δ_{C} 179.0) showed correlations with the two H-24, H-25 and 3H-26 (δ_{H} 1.26, d, $J = 7.40$ Hz) resonances. The molecular formula indicated two rings in the sidechain due to the C-17,23 ether and 27,23-lactone. The NOESY spectrum showed correlations between the 3H-19/3H-18 and 3H-19/H-29 (δ_{H} 4.02) resonances and H-5/3H-28 resonances as expected. Correlations seen between the 3H-18/H-16 β /H-20 resonances confirmed the configuration at C-17 as *S* and that H-20 was β . Correlations

Table 1 ^1H NMR (500 MHz) chemical shifts for compounds 1, 2, 2Ac, 3, 4, 5 (CDCl_3)

No	1	2	2Ac	3	4	5
1 α	1.57	1.21	1.38	1.98	1.22	1.20
1 β	2.07	1.74	1.82	1.76	1.75	1.76
2 α	2.34	1.78	1.73	2.46	1.78	1.75
2 β	2.44	1.66	1.83	2.58	1.82	1.81
3	—	3.76 ^a	4.89, dd, $J = 11.60, 4.10$	—	3.47, dd, $J = 9.90, 4.10$	3.44, dd, $J = 12.00, 4.60$
4	2.28	—	—	—	—	—
5	1.40	1.22	1.64	1.86	1.18, m	1.18, m
6 α	1.46	1.48	1.63	1.50	1.38	1.36
6 β	1.78	1.77	1.75	1.69	1.82	1.83
7 α	2.04	1.98	2.03	2.05	2.48	2.47
7 β	2.11	1.98	—	2.05	2.56	2.26
11 α	2.13	1.99	2.17	2.07	2.13	2.13
11 β	2.21	1.99	—	2.07	2.13	2.13
12 α	1.54	1.49	1.52	2.20	1.70	1.72
12 β	2.25	2.20	2.26	2.20	2.10	2.11
15 α	1.45	1.42	1.45	1.38	—	—
15 β	1.75	1.72	1.76	1.68	—	—
16 α	2.12	2.07	2.14	1.84	5.48 s	5.61, s
16 β	2.72, d, $J = 6.40$	2.76, d, $J = 6.40$	2.73	2.50	—	—
18	1.00, 3H, s	0.94, 3H, s	0.96, s	0.92, s	0.94, s	0.94, s
19	1.20, 3H, s	0.96, 3H, s	1.06, s	1.05, s	0.95, s	0.95, s
20	2.49	2.47	2.50	2.28	2.80	2.87
21	1.13, 3H, d, $J = 6.80$	1.10, d, $J = 6.60$	1.14, d, $J = 6.60$	1.05, d, $J = 6.80$	1.13, d, $J = 6.90$	1.13, d, $J = 6.50$
22 α	2.75, d, $J = 6.40$	2.03	2.04	2.72, dd, $J = 6.70, 13.50$	1.64	1.49
22 β	2.03	2.72	2.77, d, $J = 6.20$	1.78	1.73	2.04
23	—	—	—	—	3.28, bt ^a	3.97, d, $J = 11.30$
24 α	—	—	—	2.47	3.31, m ^a	—
24 β	—	—	—	2.00	—	—
25 α	—	—	—	2.97	1.43 ^a	2.44 ^a
25 β	—	—	—	—	1.59 ^a	2.53 ^a
26	—	—	—	1.26, d, $J = 7.40$	0.99, t, $J = 7.80$	1.11, t, $J = 7.40$
28A	1.02, 3H, d, $J = 6.7$	3.74 ^a	4.04, d, $J = 12.00$	1.26	1.26, s	1.26, s
28B	—	4.37, d, $J = 11.60$	4.27, d, $J = 12.00$	—	—	—
29A	—	3.81 ^a	4.31, d, $J = 12.20$	3.45, d, $J = 11.20$	3.38, d, $J = 10.90$	3.35, d, $J = 10.60$
29B	—	4.14, d, $J = 11.60$	4.40, d, $J = 12.20$	4.02, d, $J = 11.20$	4.23, d, $J = 10.90$	4.23, d, $J = 10.60$
30	1.10, 3H, s	1.09, s	1.11, s	1.05, s	1.20, s	1.21, s
COCH ₃	—	—	2.2, 2.06 s, 2.08 s	—	—	—

^a Superimposed resonances, J not clear.



seen between the 3H-26 and two H-22 resonances confirmed the configuration at C-23 as *S*. Compound **3** was identified as (17*S*,23*S*)-17 α ,23-epoxy-29-hydroxylanosta-8-en-3-on-27,23-olide.

The HREIMS of compound **4** showed a $[M + Na]^+$ peak at m/z 497.3225 which corresponded to a molecular formula of $C_{29}H_{46}O_5$ for the compound. The oxymethine C-3 resonance (δ_C 80.8), showed correlations in the HMBC spectrum with the H-5 ($J = 10.9$ Hz 1.18), the 3H-28 (δ_H 1.26) and the two H-29 (δ_H 3.35, d, δ_H 4.23, d, both $J = 10.9$ Hz) resonances, indicating the presence of an oxymethylene group at C-29 as in **3**. Correlations seen in the NOESY spectrum between the H-3 (δ_H 3.47, dd, $J = 9.90, 4.10$ Hz), 3H-28 and H-5 resonances, confirmed that H-3 was α , and a correlation was noted between the two H-29 and 3H-19 resonance (δ_H 0.95). The structure of ring D differed from that of the previous compounds having an α,β -unsaturated ring ketone, showed by resonances at δ_C 186.6 (C-17), δ_C 123.0 (C-16) and δ_C 210.1 (C-15).²⁴ The C-17 resonance showed correlations in the HMBC spectrum with the H-16 (δ_H 5.61), 3H-18 (δ_H 0.94), H-20 (δ_H 2.80), 3H-21 (δ_H 1.13, d, $J = 6.9$ Hz), and two H-22 (δ_H 1.64 and δ_H 1.73) resonances, and the C-15 resonance showed correlations with the 3H-30 (δ_H 1.20) and H-16 resonances. The COSY spectrum showed coupling between the two H-22, and the oxymethine H-23 resonance (δ_H 3.28), between H-23 and the oxymethine H-24 resonance (δ_H 3.31) and between the H-24/2H-25 (δ_H 1.43, 1.59) and 2H-25/3H-26 (δ_H 0.99, t, $J = 7.80$) resonances.

The configuration at C-23 of the co-isolated **3** and **10** have been determined unequivocally as *S*, so it was assumed that if compounds like **4** are their precursors, the configuration at C-23 should be the same (*S*). An attempt was made to determine the configuration at C-24 of the side chain vic-diol employing acyclic 1,2-diol complexation with $[Mo_2(OAc)_4]$ and subsequent CD measurements.²⁵ However, results were inconclusive. Thus **4** was identified as 3 β ,23*S*,24*e*,29-tetrahydroxy-27-norlanosta-8-16-diene-15-one.

The HREIMS of compound **5** showed $[M + Na]^+$ at m/z 495.3071 which corresponded to the molecular formula $C_{29}H_{44}O_5$ for the compound. The C-24 hydroxyl group in compound **4** was oxidised to a keto group (δ_C 212.6) in compound **5**. Compound **5** was identified as 3 β ,23*S*,29-trihydroxy-27-norlanosta-8-16-diene-15,24-dione, which has been prepared previously as a derivative of eucosterol but incomplete 1H NMR and no ^{13}C NMR data was provided.²⁴

The HRMS of compound **6Ac** gave an $[M + Na]^+$ ion at m/z 909.4254 indicating the molecular formula of $C_{47}H_{66}O_{16}$ for this compound. The FTIR spectrum for this compound showed a broad absorption at 1749 cm^{-1} for the carbonyl groups, 2937 cm^{-1} and 2868 cm^{-1} for the aliphatic CH stretches and 1222 cm^{-1} for a C–O stretch. The ^{13}C NMR spectrum indicated that compound **6Ac** was the acetylated form of the C-3 β , β -D-glucoside derivative of compound **5**. The 1H NMR spectrum showed acetylation had occurred at the C-29 and the C-23 hydroxy groups by downfield shifts of the H-23 and two H-29 proton resonances compared to those of **5** (Tables 1 and 3). The C-3 (δ_C 90.1) resonance showed a correlation in the HMBC spectrum with an anomeric proton resonance at δ_H 4.54 and the COSY

Table 2 ^{13}C NMR (125 MHz) chemical shifts for compounds **1**, **2**, **2Ac**, **3**, **4**, **5** (CDCl₃)

No	1	2	2a	3	4	5
1	37.1 CH ₂	35.3 CH ₂	35.3 CH ₂	35.6 CH ₂	35.2 CH ₂	35.2 CH ₂
2	38.0 CH ₂	27.6 CH ₂	23.9 CH ₂	29.0 CH ₂	28.2 CH ₂	28.2 CH ₂
3	213.1 C	77.9 CH	74.0 CH	221.0 C	80.9 CH	80.8 CH
4	45.2 CH	46.1 C	44.1 C	51.3 C	43.0 C	42.9 C
5	49.7 CH	47.5 CH	44.5 CH	52.1 CH	51.2 CH	51.2 CH
6	22.1 CH ₂	18.8 CH ₂	19.4 CH ₂	19.1 CH ₂	18.4 CH ₂	18.1 CH ₂
7	25.4 CH ₂	26.3 CH ₂	26.8 CH ₂	26.2 CH ₂	27.4 CH ₂	27.7 CH ₂
8	135.8 C	134.8 C	135.2 C	132.6 C	133.6 C	133.4 C
9	133.1 C	134.6 C	134.5 C	136.3 C	135.8 C	136.1 C
10	36.8 C	36.9 C	37.1 C	37.0 C	37.5 C	37.6 C
11	21.5 CH ₂	20.8 CH ₂	20.7 CH ₂	21.0 CH ₂	20.6 CH ₂	20.4 CH ₂
12	24.7 CH ₂	24.6 CH ₂	24.6 CH ₂	25.0 CH ₂	23.3 CH ₂	23.2 CH ₂
13	50.9 C	50.8 C	50.9 C	48.7 C	51.2 C	51.2 C
14	49.0 C	49.0 C	49.0 C	50.7 C	57.3 C	57.3 C
15	31.5 CH ₂	31.5 CH ₂	31.5 CH ₂	31.9 CH ₂	210.2 C	210.1 C
16	39.3 CH ₂	39.4 CH ₂	39.3 CH ₂	37.3 CH ₂	123.0 CH	123.0 CH
17	98.4 C	98.6 C	98.4 C	98.7 C	186.6 C	185.8 C
18	18.2 CH ₃	17.9 CH ₃	18.3 CH ₃	18.9 CH ₃	28.8 CH ₃	28.8 CH ₃
19	17.7 CH ₃	19.8 CH ₃	19.3 CH ₃	19.9 CH ₃	19.7 CH ₃	19.8 CH ₃
20	41.9 CH	41.9 CH	41.9 CH	44.0 CH	30.1 CH	30.2 CH
21	17.9 CH ₃	17.9 CH ₃	17.9 CH ₃	18.8 CH ₃	21.7 CH ₃	21.7 CH ₃
22	39.2 CH ₂	39.2 CH ₂	39.2 CH ₂	32.0 CH ₂	39.4 CH ₂	39.6 CH ₂
23	177.1 C	177.4 C	177.4 C	113.4 C	71.9 CH	74.3 CH
24	—	—	—	45.1 CH ₂	76.9 CH	212.6 C
25	—	—	—	35.8 CH	26.7 CH ₂	31.4 CH ₂
26	—	—	—	15.1 CH ₃	10.2 CH ₃	7.8 CH ₃
27	—	—	—	179.0 C	—	—
28	11.7 CH ₃	63.9 CH ₂	62.7 CH ₂	22.1 CH ₃	22.5 CH ₃	22.5 CH ₃
29	—	71.4 CH ₂	63.8 CH ₂	66.1 CH ₂	64.5 CH ₂	64.5 CH ₂
30	25.9 CH ₃	25.8 CH ₃	25.7 CH ₃	26.2 CH ₃	29.6 CH ₃	29.6 CH ₃
1-OAc	—	—	21.4 CH ₃	—	—	—
2-OAc	—	—	21.3 CH ₃	—	—	—
3-OAc	—	—	21.2 CH ₃	—	—	—
1-OAc	—	—	170.4 C	—	—	—
2-OAc	—	—	170.9 C	—	—	—
3-OAc	—	—	171.2 C	—	—	—

and HSQC spectra enabled assignment of the sugar resonances. The correlations seen in the NOESY spectrum between H-1'/H-3' and H-2'/H-4' resonances, and the coupling constants $J_{1',2'}$ (8.0 Hz) and $J_{3',4'}$ (9.6 Hz) confirmed that the sugar present was acetylated β -D-glucose.²⁶ Thus, compound **6Ac** is 23*S*,29-diacetoxy-3 β -[2',3',4',6'-tetra-*O*-acetyl-*O*- β -D-glucopyranoside]-27-norlanosta-8,16-diene-15,24-dione.

Compound **7Ac** was identified as 23*S*,29-diacetoxy-3 β -[2',3',4'-tri-*O*-acetyl-*O*- β -D-glucopyranoside-(1'' \rightarrow 6')-2'',3'',4''-tri-*O*-acetyl- β -D-arabinopyranosyl]-27-norlanosta-8,16-diene-15,24-dione. The HRMS spectrum for compound **7Ac** gave a $[M + Na]^+$ ion at m/z 1125.4865 indicating a molecular formula of $C_{56}H_{78}O_{22}$ for this acetylated compound. The aglycone part of compound was found to be the same as for compounds **5Ac** and **6Ac**. However, in addition to the acetylated β -D-glucose at C-3 β , the molecular formula indicated an additional acetylated pentose sugar and this was confirmed by the ^{13}C NMR spectrum which displayed two characteristic anomeric carbon resonances at δ_C 102.7 in table (C-1') and δ_C 101.1 in table (C-1'') with





Table 3 ^1H NMR (950 MHz) chemical shifts for compounds **6Ac–14Ac** (CDCl_3)

No	6Ac	7Ac	8Ac	9Ac	10Ac	11Ac	12Ac	13Ac	14Ac
1 α	1.21	1.21	1.21	1.25	1.20	1.24	1.22	1.23	1.22
1 β	1.82	1.87	1.79	1.86	1.79	1.85	1.75	1.81	1.82
2 α	1.76	1.76	1.93	2.00	1.99	1.99	1.80	1.74	2.27
2 β	1.92	1.90			2.06	2.07	1.97	1.90	2.65
3	3.20, dd, $J = 4.00$, 11.80 Hz	3.22, dd, $J = 3.20$, 12.30	3.21, dd, $J = 4.10$, 11.90	3.21, dd, $J = 4.00$, 12.20	3.19, dd, $J = 4.00$, 11.60	3.20	3.19, dd, $J = 4.00$, 11.80	3.21, dd, $J = 4.30$, 12.00	3.16, dd, $J = 4.30$, 12.00
4	—	—	—	—	—	—	—	—	—
5	1.17	1.15	1.14	1.17	1.16	1.12	1.14	1.14	1.09
6 α	1.56	1.55	1.60	1.60	1.61	1.61	1.85	1.54	1.56
6 β	1.88	1.88	1.78	1.83	1.80	1.81	1.98	1.84	1.83
7 α	2.42	2.42	1.73	1.75	1.76	1.77	1.78	1.75	1.70
7 β	2.58	2.57	1.91	1.92	1.93	1.89	1.92	1.91	1.86
11	2.15	2.16	2.07	2.07	1.98	2.10	2.04	2.11	1.92
12 α	1.65	1.65	2.17	2.16	1.43	1.46	1.99	1.60	1.58
12 β	2.13	2.11			2.22	2.22	2.08	2.34	2.34
15 α	—	—	1.32	1.37	1.35	1.38	—	—	—
15 β	—	—	1.66	1.69	1.66	1.68	—	—	—
16 α	5.60, s	5.60, s	1.79	1.81	1.63	1.70	2.22, d, $J = 19.2$	2.22, d, $J = 19.20$	2.20, d, $J = 19.20$
16 β	—	—	2.50	2.52	1.99	2.00	2.77, d, $J = 19.2$	2.77, d, $J = 19.20$	2.76, d, $J = 19.20$
18	0.84, s	0.85, s	0.90	0.90, s	0.91	0.88, s	0.94, s	0.94	0.93, s
19	1.02, s	1.02, s	1.00	0.99, s	1.00	0.93, s	1.02, s	1.02, s	1.02, s
20	2.67	2.67	2.24	2.28	2.18	2.21	2.29	2.26	2.24
21	1.15, d, $J = 7.20$	1.15, d, $J = 6.60$	1.05, d, $J = 6.90$	1.07, d, $J = 6.70$	1.06, d, $J = 7.70$	1.13, d, $J = 6.50$	1.11, d, $J = 6.70$	1.11, d, $J = 6.80$	1.12, d, $J = 6.80$
22 α	1.84	1.85	1.76	1.79	1.81	1.84	1.89	1.92	1.86
22 β	2.03	2.06	2.71, dd, $J = 6.50$, 13.90	2.74	1.99	2.00	1.94	1.99	1.99
23	4.86, dd, $J = 2.70$, 10.50 Hz	4.87, dd, $J = 3.00$, 10.90	—	—	4.56 ^a	4.54	4.69, dd, $J = 8.70$, 10.40	4.68, dd, $J = 8.70$, 9.90	4.67, dd, $J = 9.00$, 9.40
24 α	—	—	1.99	2.03	—	—	—	—	—
24 β	—	—	2.47	2.50	—	—	—	—	—
25 α	2.45	2.41			2.56, q, $J = 7.30$	2.57, q, $J = 7.40$	2.48, q, $J = 7.00$	2.49, q, $J = 7.30$	2.48, q, $J = 7.30$
25 β	2.51	2.57	2.94	2.98	1.08, t, $J = 7.30$	1.11, t, $J = 7.40$	1.07, t, $J = 7.20$	1.07, t, $J = 7.30$	1.07, t, $J = 7.30$
26	1.07, t, $J = 7.30$ Hz	1.07, t, $J = 6.50$	1.25, d, $J = 7.10$	1.28, d, $J = 6.70$	—	—	—	—	—
27	—	—	—	—	—	—	—	—	—
28	1.09, s	1.09, s	1.07	1.10, s	1.08	1.07, s	1.08, s	1.08, s	1.05, s
29 α	4.17 ^a	4.16 ^a	4.16 ^a	4.16 ^a	4.17 ^a	4.16	4.23 ^a	4.16, d, $J = 11.80$	4.15, d, $J = 11.90$
29 β	4.24 ^a	4.25 ^a	4.23 ^a	4.25 ^a	4.24 ^a	4.25	4.15 ^a	4.24, d, $J = 11.80$	4.21, d, $J = 11.90$



Table 3 (Contd.)

No	6Ac	7Ac	8Ac	9Ac	10Ac	11Ac	12Ac	13Ac	14Ac
30	1.19, s	1.19, s	1.00	1.01, s	1.26	1.21, s	1.37, s	1.37, s	1.35, s
Glu 1'	4.54, d, <i>J</i> = 8.00 Hz	4.53, d, <i>J</i> = 8.00	4.54, d, <i>J</i> = 8.00	4.52, d, <i>J</i> = 8.20	4.56, d, <i>J</i> = 8.20	4.52, d, <i>J</i> = 8.10	4.54, d, <i>J</i> = 7.90	4.52, d, <i>J</i> = 8.70	4.36, d, <i>J</i> = 8.10
	4.98, dd, <i>J</i> = 9.60, 8.00 Hz	4.95, dd, <i>J</i> = 9.70, 8.00	5.00, dd, <i>J</i> = 8.20, 9.40 Hz	4.95, dd, <i>J</i> = 8.20, 9.70	5.00, dd, <i>J</i> = 8.10, 9.50	4.90	5.00, dd, <i>J</i> = 8.10, 9.80	4.94, dd, <i>J</i> = 8.20, 9.40	4.94, dd, <i>J</i> = 8.10, 9.60
3'	5.18, t, <i>J</i> = 9.60 Hz	5.19, t, <i>J</i> = 9.70	5.19, t, <i>J</i> = 9.50	5.18, t, <i>J</i> = 9.70	5.19, t, <i>J</i> = 9.40	5.18	5.18, t, <i>J</i> = 9.80	5.17, t, <i>J</i> = 9.40	3.78, t, <i>J</i> = 9.30
4'	5.06, t, <i>J</i> = 9.60 Hz	4.93, t, <i>J</i> = 9.70	5.07, t, <i>J</i> = 9.70 Hz	4.93, t, <i>J</i> = 9.70	5.06, t, <i>J</i> = 9.60	4.93	5.05, t, <i>J</i> = 9.80	5.06, t, <i>J</i> = 9.50	4.80, t, <i>J</i> = 9.30
5'	3.68, m	3.68, m	3.66, m	3.69, m	3.66, m	3.68	3.65, m	3.66, m	3.60, m
6'A	4.12, m	3.65, m	4.23 ^a	3.65, m	4.22 ^a	3.63	4.13	3.65, m	3.62, m
6'B	4.20, m	3.84, m	4.12 ^a	3.82, m	4.12 ^a	3.82	4.22	3.82, m	3.79, m
Arab 1''		4.50, d, <i>J</i> = 6.60	—	4.50, d, <i>J</i> = 6.70	—	4.49, d, <i>J</i> = 6.10	—	4.49, d, <i>J</i> = 6.80	4.48, d, <i>J</i> = 6.60
2''		5.16, dd, <i>J</i> = 6.60, 8.80	—	5.14, dd, <i>J</i> = 6.70, 9.00	—	5.15	—	5.14, dd, <i>J</i> = 6.80, 8.90	5.13, dd, <i>J</i> = 6.60, 8.80
3''		5.03, dd, <i>J</i> = 3.70, 8.80	—	5.03, dd, <i>J</i> = 3.60, 9.07	—	5.03	—	5.01, dd, <i>J</i> = 3.50, 8.90	5.00, dd, <i>J</i> = 3.50, 8.90
4''		5.25, brs	—	5.25, brs	—	5.26	—	5.28, brs	5.24, brs
5''α		4.05	—	4.03	—	4.03	—	4.03, m	4.01, dd, <i>J</i> = 3.60, 12.90
5''β		3.63	—	3.62	—	3.60	—	3.61, dd, <i>J</i> = 3.80, 12.90	3.60, m
Xyl 1'''			—	—	—	—	—	—	4.53, d, <i>J</i> = 6.20
2'''			—	—	—	—	—	—	4.75, dd, <i>J</i> = 6.20, 7.80
3'''			—	—	—	—	—	—	5.03, t, <i>J</i> = 7.80
4'''			—	—	—	—	—	—	4.85, m
5'''α			—	—	—	—	—	—	3.34, dd, <i>J</i> = 7.90, 12.00
5'''β			—	—	—	—	—	—	4.06, dd, <i>J</i> = 4.40, 12.00
OAc	2.05, 2.02, 2.12, 2.10, 2.08, 2.02	1.98, 2.02, 2.02, 2.04, 2.05, 2.08, 2.12, 2.13	2.00, 2.02, 2.02, 2.05, 2.08	1.98, 2.02, 2.02, 2.04, 2.04, 2.07, 2.08	1.99, 2.02, 2.02, 2.04, 2.08	1.98, 2.02, 2.02, 2.04, 2.07, 2.08	1.98, 2.02, 2.02, 2.04, 2.07, 2.08	1.98, 2.02, 2.02, 2.04, 2.04, 2.07, 2.08	1.98, 2.02, 2.02, 2.04, 2.04, 2.07, 2.08, 2.13, 2.12

^a Overlapped signals.

corresponding anomeric proton resonances at δ_{H} 4.53 (d, $J = 8.0$ Hz) and δ_{H} 4.50 (d, $J = 6.6$ Hz). The anomeric proton resonance at δ_{H} 4.50, assigned as H-1'', was seen to correlate in the HMBC spectrum to C-6' of β -D-glucose of C-6' indicating a 1'' \rightarrow 6' linkage. The COSY and HSQC spectra were again used to assign the pentose sugar resonance and the NOESY spectrum and coupling constants were used to confirm whether the pentose was xylose or arabinose. The coupling constants of $J_{1'',2''} = 6.60$ Hz, $J_{2'',3''} = 8.80$ Hz and $J_{3'',4''} = 3.70$ Hz indicated that H-1''/H-2'' and H-2''/H-3'' are *trans*-diaxial and that H-3'' and H-4'' have an axial-equatorial relationship. Hence, the sugar was identified as β -D-arabinose and this was supported by correlations seen in the NOESY spectrum between the H-1''/H-3'', H-3''/H-4'', H-4''/H_B-5'' and H-1''/H_A-5'' resonances.

Compound **8Ac** was identified as (17*S*,23*S*)-29-acetoxy-23,17-epoxy-3 β -[2',3',4',6'-tetra-*O*-acetyl-*O*- β -D-glucopyranoside]-lanost-8-en-27,23-olide, the 3 β - β -D-glucoside derivative of compound **3**. The molecular ion could not be observed in the LCMS spectrum for compound **8Ac**, but the structure could be determined from NMR studies. The ^{13}C NMR chemical shifts for the aglycone part were similar to those of compound **3**, except that the C-3 keto group carbon was replaced by an oxymethine resonance (δ_{C} 90.4) and the C-29 hydroxy group had been acetylated. The HMBC spectrum showed a correlation between the H-3 α proton resonances (δ_{H} 3.21, d, $J = 4.1$, 11.9 Hz) and an anomeric carbon resonance (δ_{C} 103.2), which corresponded to the proton resonance at δ_{H} 4.54 (d, $J = 8.0$ Hz). The coupling constants and NOESY correlations of the sugar present were the same as those of compound **6Ac**, indicating that the sugar present was acetylated β -D-glucose.

Compound **9Ac** was identified as (17*S*,23*S*)-29-acetoxy-23,17-epoxy-3 β -[2',3',4'-tri-*O*-acetyl-*O*- β -D-glucopyranoside-(1'' \rightarrow 6')-2'',3'',4''-tri-*O*-acetyl-*O*- β -D-arabinopyranosyl]-lanost-8-en-27,23-olide. The HRMS for compound **9Ac** gave a $[\text{M} + \text{Na}]^+$ ion at m/z 1097.4880, which indicated the molecular formula of $\text{C}_{55}\text{H}_{78}\text{O}_{21}$ for this compound. The ^{13}C NMR spectrum displayed fifty-five carbon resonances, and showed that the aglycone structure was the same as for **8Ac**, but that an extra β -D-arabinose sugar was present and linked as determined for **7Ac**.

Compound **10Ac** was identified as (17*S*,23*S*)-29-acetoxy-23,17-epoxy-3 β -[2',3',4',6'-tetra-*O*-acetyl-*O*- β -D-glucopyranoside]-27-norlanost-8-en-24-one. The LCMS spectrum for compound **10Ac** gave a $[\text{M}]^+$ ion at m/z 830.4421, which indicated the molecular formula of $\text{C}_{45}\text{H}_{66}\text{O}_{14}$ for this compound. The aglycone part of the molecule was found to be the 29-acetyl derivative of the co-isolated 15-deoxyeucosterol. The C-17 (δ_{C} 97.3) resonance showed correlations in the HMBC spectrum with the 3H-19 (δ_{H} 1.00), H-20 (δ_{H} 1.26), 3H-21 (δ_{H} 1.06, d, $J = 7.0$ Hz), two H-22 (δ_{H} 1.81 and δ_{H} 1.99) and H-23 (δ_{H} 4.56) resonances. A ketone carbon resonance at δ_{C} 213.3 was assigned as C-24 as it showed correlations with the two H-22, H-23, two H-25 (δ_{H} 2.56, q, $J = 7.3$ Hz) and 3H-26 (δ_{H} 1.08, t, $J = 7.3$ Hz) resonances. The H-29 protons occurred as a pair of doublets (δ_{H} 4.17, δ_{H} 4.24) and H-3 α at δ_{H} 3.19 (dd, $J = 4.0$, 11.6 Hz). The C-3 resonance showed a correlation in the HMBC spectrum with the anomeric proton resonance at δ_{H} 4.56. The chemical shifts, coupling constants and correlations seen in the NOESY spectrum of the sugar

present were the same as those of the acetylated β -D-glucose in compounds **6Ac** and **8Ac**. Oxidative cleavage of the C-23/C-24 bond of **10Ac** would lead to the γ -lactone structure seen in **1** and **2**.

Compound **11Ac** was identified as (17*S*,23*S*)-29-acetoxy-23,17-epoxy-3 β -[2',3',4'-tri-*O*-acetyl-*O*- β -D-glucopyranoside-(1'' \rightarrow 6')-2'',3'',4''-tri-*O*-acetyl-*O*- β -D-arabinopyranosyl]-27-norlanost-8-en-24-one. The expected $[\text{M} + \text{Na}]^+$ peak at m/z 1069.4984 for this compound was not observed in the LCMS spectrum, however, a base peak at m/z 587.1579, which was attributed to the acetylated disaccharide fragment $[\text{C}_{23}\text{H}_{31}\text{O}_{16}\text{Na} + \text{H}]^+$ was observed. The extra hydrogen is unusual, but was also found in compound **14Ac**. It is suggested that this hydrogen comes from the triterpenoid system, solvent or electrospray buffer system. As with compounds **6Ac/7Ac** and **8Ac/9Ac**, compound **11Ac** differed from **10Ac** in having an extra acetylated β -D-arabinose group at C-6'. This was confirmed using 2D NMR correlations and ^1H NMR coupling constants.

The HRMS spectrum for compound **12Ac**, (17*S*,23*S*)-29-acetoxy-23,17-epoxy-3 β -[2',3',4',6'-tetra-*O*-acetyl-*O*- β -D-glucopyranoside]-27-norlanost-8-ene-15,24-dione, gave a $[\text{M} + \text{Na}]^+$ ion at m/z 867.4147, which indicated the molecular formula of $\text{C}_{45}\text{H}_{64}\text{O}_{15}$ for this compound. **12Ac** was the 15-keto derivative of **10Ac**. The C-15 (δ_{C} 215.6) resonance showed correlations in the HMBC spectrum with the 3H-30 (δ_{H} 1.37) and pair of H-16 (2.22, 2.77, ea d, $J = 19.2$ Hz) proton resonances.

Compound **13Ac** was identified as the 15-keto derivative of **11Ac**, (17*S*,23*S*)-29-acetoxy-23,17-epoxy-3 β -[2',3',4'-tri-*O*-acetyl-*O*- β -D-glucopyranoside-(1'' \rightarrow 6')-2'',3'',4''-tri-*O*-acetyl-*O*- β -D-arabinopyranosyl]-27-norlanost-8-ene-15,24-dione). The HRMS spectrum gave a $[\text{M} + \text{Na}]^+$ ion at m/z 1083.4739 which corresponded to the molecular formula of $\text{C}_{54}\text{H}_{76}\text{O}_{21}$ for this compound. The NMR spectra showed one fewer methylene group but an extra C-15 ketone carbonyl carbon resonance (δ_{C} 215.6) which showed correlations in the HMBC spectrum with the 3H-30 (δ_{H} 1.37) and two H-16 (δ_{H} 2.22 and 2.77, ea d, $J = 19.2$ Hz) resonances. The identification of the sugars was done as for the previous disaccharides isolated.

Compound **14Ac** was identified as the acetylated trisaccharide of eucosterol, (17*S*,23*S*)-29-acetoxy-23,17-epoxy-3 β -[2',3',4'-tri-*O*-acetyl-*O*- β -D-glucopyranoside-(1'' \rightarrow 6')-2'',3'',4''-tri-*O*-acetyl-*O*- β -D-arabinopyranosyl-(1''' \rightarrow 3')-2''',3''',4'''-tri-*O*-acetyl-*O*- β -D-xylopyranosyl]-27-norlanost-8-ene-15,24-dione. The expected $[\text{M} + \text{Na}]^+$ peak at m/z 1276.5513 for this compound was not observed in the LCMS spectrum. However, a base peak at m/z 803.2235, which was attributed to the acetylated trisaccharide fragment $[\text{C}_{32}\text{H}_{43}\text{O}_{22}\text{Na} + \text{H}]^+$ was observed, similar to that seen in the MS of compound **11Ac**. The ^{13}C NMR spectrum displayed sixty-three carbons resonance and the aglycone part was found to be the same as in compounds **12Ac** and **13Ac**. The ^{13}C NMR spectrum displayed three anomeric carbon resonances at δ_{C} 100.3, δ_{C} 101.7 and δ_{C} 102.7, which corresponded to the proton resonances at δ_{H} 4.48 (d, $J = 6.6$ Hz), δ_{H} 4.53 (d, $J = 6.2$ Hz) and δ_{H} 4.36 (d, $J = 8.1$ Hz), respectively, confirming that compound **14Ac** possessed three sugar groups. The C-3 resonance was seen to correlate in the HMBC spectrum with the resonance at δ_{H} 4.36, which was assigned as H-1'. This was used as a starting point to assign the



chemical shifts of the first sugar. The COSY and HSQC spectrum enabled all ^1H and ^{13}C NMR resonances for the sugar at C-3 β to be assigned and the large coupling constants ($J_{1',2'} = 8.1$ Hz, $J_{3',4'} = 9.30$ Hz) confirmed the *trans*-diaxial relationships between H-1/H-2 and H-3/H-4 indicating the presence of an acetylated β -D-glucose unit. This was supported by correlations seen in the NOESY spectrum between the H-1' and H-3', H-1' and H-5' and H-2' and H-4' resonances.

As in previous compounds, the C-6' resonance showed a correlation in the HMBC spectrum with the anomeric proton resonance at δ_{H} 4.48 (H-1'') of β -D-arabinose. All resonances for this sugar could be assigned and the coupling constants of $J_{1'',2''} = 6.6$ Hz and $J_{3'',4''} = 3.5$ Hz indicated *trans*-diaxial and axial-equatorial relationships respectively, confirmed by correlations seen in the NOESY spectrum between the H-1''/H-3'', H-3''/H-4'', H-4''/H-5'' β and between H-1''/H-5'' α resonances identified the sugar. The C-3' resonance was seen to correlate with the anomeric proton resonance at δ_{H} 4.53 of the pentose sugar in the HMBC spectrum, which was assigned as H-1''', indicating the third sugar was linked to β -D-glucose at C-3'. The $J_{1''',2'''} = 6.20$ Hz and $J_{3''',4'''} = 7.80$ Hz indicated that H-1'''/H-2''' and H-3'''/H-4''' both have *trans*-diaxial relationships, hence, the sugar was identified as β -D-xylose. This was confirmed by correlations seen in the NOESY spectrum between the H-1'''/H-3''' and H-4'''/H-2''' resonances.

(R)-5,7-Dihydroxy-8-methoxy-3-(4'-methoxybenzyl)-4-chromanone, eucosterol, (17S,23S)-23,17-epoxy-3 β ,28,29-trihydroxy-27-norlanost-8-en-24-one, **1**, **2Ac**, **3**, **4** and **5** were evaluated against the NCI 60 developmental Therapeutics Program 60 cancer cell line screen at a single dose of 10^{-5} M. Details of the methodology of NCI-60 Human Tumour Cell Line Screen are described at <http://dtp.nci.nih.gov/branches/btb/ivclsp.html>. None of the compounds showed sufficient activity to progress to the five-dose assay although **5** showed 72.5% inhibition of the H522 non-small lung cell line. One dose mean graphs for the compounds screened are provided in the ESI (S.5 †).

This investigation produced typical *Eucomis*-type constituents, homoisoflavonoids and lanostane triterpenoid derivatives. The variability of constituents isolated from the Hyacinthaceae has been noted by us previously.⁴⁻⁶ Only two of the compounds isolated in this work had been reported to occur in this species previously, the common homoisoflavonoids 3,5,7-trihydroxy-3-(4'-methoxybenzyl)-4-chromanone⁷ and 5,7-dihydroxy-3-(4'-methoxybenzyl)-4-chromanone.⁸ Compounds **3**, **8** and **9** had the same spirocyclic triterpenoid skeleton as scillasponin A,¹⁰ but instead of being a pentaglycoside, occurred as the free triterpenoid (**3**) or as the mono- (**8**) or di-glycosides (**9**). Compounds **10–14** differed from related compounds isolated previously in that the C-28 methyl group was not oxidised, and the sugars differed. Rhamnopyranosyl sugars found in compounds isolated previously from the Japanese collection⁹ were not found in this collection from its natural habitat.

Experimental section

General experiment procedures

1D and 2D NMR spectra were recorded in CDCl_3 on a 500 MHz Bruker AVANCE NMR instrument at room temperature at the

University of Surrey and those of the acetylated triterpenoid glycosides were recorded on a 950 MHz Bruker AVANCE NMR instrument at the David H. Murdock Research Institute (DHMRI), North Carolina Research Campus (NCRC) in Kannapolis, North Carolina. The chemical shifts (δ) are expressed in ppm and were referenced to the solvent resonances at 7.26 and 77.23 ppm for CDCl_3 , 4.87 and 49.15 for CD_3OD for ^1H and ^{13}C NMR analysis respectively. The ESI mass spectra were recorded on a Bruker MicroToF mass spectrometer using an Agilent 1100 HPLC to introduce samples (University of Oxford). The optical rotations were determined in CHCl_3 on a JASCO P-1020 polarimeter. The FTIR spectra were recorded using a Perkin-Elmer (2000) spectrometer.

Plant material

Bulbs of *Eucomis bicolor* (1.5 kg) were collected from the Drakensberg foothills, South Africa during January 2008. A voucher specimen has been lodged for verification purposes (N. Crouch 1157, NH).

Extraction and isolation of compounds

The whole bulbs of *Eucomis bicolor* (1.5 kg) were air-dried, chopped into smaller pieces and extracted with methylene chloride and methanol using a shaker for 48 h at room temperature. The extract was then filtered and the solvent was removed under reduced pressure to yield the methylene chloride extract (26.31 g) and the methanol extract (104.87 g). Column chromatography over silica gel (Merck 9385) using a 5 cm diameter gravity column, collecting fractions (75 ml each) using a methylene chloride/methanol step gradient starting with 100% methylene chloride and gradually increasing the methanol concentration to 100%. Final purification was undertaken using 1 cm diameter gravity column. Full details are provided in the ESI (S.2 †).

The methylene chloride extract yielded (R)-5,7-dihydroxy-8-methoxy-3-(4'-methoxybenzyl)-4-chromanone (6.5 mg), 3,5,7-trihydroxy-3-(4'-methoxybenzyl)-4-chromanone (7.2 mg) and (R)-5,7-dihydroxy-3-(4'-methoxybenzyl)-4-chromanone (7.9 mg), eucosterol (10.2 mg), (17S,23S)-17,23-epoxy-3 β ,28,29-trihydroxy-27-norlanost-8-en-24-one (3.2 mg), 15-deoxoeucosterol (8.2 mg) and 3-dehydro-15-deoxoeucosterol (8.2 mg) and five novel compounds, **1** (8.5 mg), compound **2** which was acetylated to give compound **2Ac** (2.8 mg), **3** (3.3 mg), **4** (3.6 mg) and **5** (2.5 mg). The methanol extract yielded (R)-5,7-trihydroxy-3-(4'-hydroxybenzyl)-4-chromanone (8.2 mg) and nine novel lanosterol glycosides which were isolated as their acetate derivatives after acetylation of a complex mixture to yield **6Ac** (1.9 mg), **7Ac** (1.2 mg), **8Ac** (1.8 mg), **9Ac** (1.1 mg), **10Ac** (2.3 mg), **11Ac** (0.8 mg), **12Ac** (2.1 mg), **13Ac** (1.7 mg) and **14Ac** (1.4 mg). A detailed isolation scheme is given in the ESI (S.2 †). Full NMR data for compounds **1–5** and acetates of **2**, and **6–14** are given in Tables 1–5 and spectra are given in the ESI (S.4 †).

(17S)-24,25,26,27,29-Pentanor-3-oxo-lanost-8-en-23,17 α -olide (**1**). Pale yellow powder; $[\alpha]_{\text{D}}^{23.6} +29.15$ (c 0.0094, CHCl_3); IR ν_{max} (NaCl) cm^{-1} 2953, 2923 cm^{-1} (C–H stretches) and 1767, 1708



Table 4 ^{13}C NMR (237 MHz) chemical shifts for compounds 6Ac–7Ac, 10Ac–12Ac (CDCl_3)

No	6Ac	7Ac	10Ac	11Ac	12Ac
1	35.8 CH_2	35.6 CH_2	36.0 CH_2	36.0 CH_2	35.9 CH_2
2	27.3 CH_2	27.2 CH_2	27.2 CH_2	27.1 CH_2	27.4 CH_2
3	90.1 CH	89.9 CH	90.3 CH	90.3 CH	90.3 CH
4	42.3 C	42.3 C	42.4 C	42.4 C	42.3 C
5	51.4 CH	51.1 CH	52.2 CH	51.9 CH	51.3 CH
6	19.5 CH_2	19.4 CH_2	19.7 CH_2	19.5 CH_2	19.6 CH_2
7	26.5 CH_2	26.3 CH_2	26.8 CH_2	26.8 CH_2	26.5 CH_2
8	133.4 C	133.3 C	134.2 C	134.3 C	133.5 C
9	136.0 C	136.0 C	135.7 C	135.7 C	135.9 C
10	37.4 C	37.4 C	37.1 C	37.0 C	37.4 C
11	20.5 CH_2	20.5 CH_2	21.0 CH_2	21.2 CH_2	20.5 CH_2
12	23.0 CH_2	22.9 CH_2	25.1 CH_2	25.1 CH_2	23.1 CH_2
13	47.8 C	47.6 C	48.7 C	48.8 C	47.6 C
14	57.9 C	57.9 C	50.9 C	50.8 C	57.9 C
15	215.6 C	215.7 C	31.9 CH_2	31.9 CH_2	215.6 C
16	51.9 CH_2	51.7 CH_2	39.9 CH_2	39.8 CH_2	52.1 CH_2
17	91.2 C	91.2 C	97.3 C	97.3 C	91.3 C
18	20.7 CH_3	20.4 CH_3	19.2 CH_3	19.5 CH_3	20.7 CH_3
19	19.1 CH_3	19.6 CH_3	18.9 CH_3	19.2 CH_3	19.0 CH_3
20	43.5 CH	43.3 CH	44.0 CH	43.7 CH	43.2 CH
21	17.4 CH_3	17.3 CH_3	17.4 CH_3	17.4 CH_3	17.3 CH_3
22	37.2 CH_2	36.7 CH_2	37.1 CH_2	37.0 CH_2	37.0 CH_2
23	81.3 CH	81.8 CH	81.8 CH	81.6 CH	81.6 CH
24	212.2 C	212.2 C	213.6 C	213.6 C	212.2 C
25	32.7 CH_2	32.5 CH_2	32.2 CH_2	32.4 CH_2	32.5 CH_2
26	7.6 CH_3	7.5 CH_3	7.8 CH_3	7.6 CH_3	7.4 CH_3
28	22.6 CH_3	22.7 CH_3	22.3 CH_3	22.5 CH_3	22.6 CH_3
29	65.7 CH_2	65.5 CH_2	65.5 CH_2	65.5 CH_2	65.8 CH_2
30	23.9 CH_3	23.7 CH_3	25.9 CH_3	25.8 CH_3	23.9 CH_3
Glu 1'	102.9 CH	102.7 CH	103.1 CH	102.9 CH	103.7 CH
2'	71.9 CH	72.7 CH	71.9 CH	71.7 CH	71.8 CH
3'	73.2 CH	80.4 CH	73.1 CH	73.1 CH	73.2 CH
4'	69.2 CH	69.2 CH	68.8 CH	69.0 CH	68.8 CH
5'	73.6 CH	73.4 CH	71.8 CH	73.3 CH	71.9 CH
6'	67.8 CH_2	67.6 CH_2	62.4 CH_2	67.3 CH_2	62.3 CH_2
Arab 1''	100.6 CH	100.3 CH	—	100.4 CH	—
2''	69.2 CH	69.0 CH	—	69.0 CH	—
3''	70.3 CH	70.0 CH	—	70.0 CH	—
4''	67.6 CH	67.4 CH	—	67.4 CH	—
5''	63.1 CH_2	62.8 CH_2	—	62.9 CH_2	—
Xyl 1'''	—	101.1 CH	—	—	—
2'''	—	70.0 CH	—	—	—
3'''	—	70.7 CH	—	—	—
4'''	—	68.6 CH	—	—	—
5'''	—	61.7 CH	—	—	—
OAc	20.6, 21.2, 21.3, 20.9, 20.9, 21.1, 20.9	20.6, 21.2, 21.3, 20.9, 21.1, 20.9, 21.2, 21.1	20.5, 20.9, 20.9, 21.0, 21.3	20.6, 21.2, 21.3, 20.9, 20.9, 21.1, 20.9	20.5, 20.7, 20.8, 20.8, 21.0
OAc	170.5, 169.7, 170.3, 170.6, 169.5, 169.4, 171.3	170.5, 169.7, 170.3, 170.6, 169.5, 169.4, 171.3, 169.1, 170.1	170.6, 169.4, 170.9, 169.6, 170.8	170.5, 169.7, 170.3, 170.6, 169.5, 169.4, 171.3	170.6, 169.4, 171.2, 169.6, 170.8

cm^{-1} (C=O stretch); HRESIMS m/z 407.2570 $[\text{M} + \text{Na}]^+$ (calc. for $\text{C}_{25}\text{H}_{36}\text{O}_3\text{Na}$ 407.2562). NMR data are given in Tables 1 and 2.

(17S)-24,25,26,27-Tetranor-3 β ,28,29-triacetoxy-lanost-8-en-23,17 α -olide (2Ac). Colourless oil; $[\alpha]_{\text{D}}^{23.6}$ 40.67 (c 0.0009, CHCl_3); IR ν_{max} (NaCl) cm^{-1} 2916, 2849 (C–H stretch) and 1778, 1738 (C=O stretch); HRESIMS m/z 581.3096 $[\text{M} + \text{Na}]^+$ (calc. for $\text{C}_{32}\text{H}_{46}\text{O}_8\text{Na}$ 581.3090). NMR data are given in Tables 1 and 2.

(17S,23S)-17 α ,23-Epoxy-29-hydroxylanost-8-en-3-on-27,23-olide (3). Colourless oil; $[\alpha]_{\text{D}}^{23.6}$ +11.25 (c 0.0032, CHCl_3); IR ν_{max} (NaCl) cm^{-1} 2923, 2853 (C–H stretch) 3467 (O–H stretch) and 1772, 1701 (C=O stretch); HRESIMS m/z 507.3078 $[\text{M} + \text{Na}]^+$ (calc. for $\text{C}_{30}\text{H}_{44}\text{O}_5\text{Na}$ 507.3086). NMR data are given in Tables 1 and 2.

3 β ,23S,24 ϵ ,29-Tetrahydroxy-27-norlanosta-8-16-diene-15-one (4). Pale Yellow oil; $[\alpha]_{\text{D}}^{23.6}$ +25.00 (c 0.0008, CHCl_3); IR ν_{max} (NaCl) cm^{-1} 2932, 2875 (C–H stretch), 3399 (O–H stretch) and



Table 5 ^{13}C NMR (237 MHz) chemical shifts for compounds **8Ac**, **9Ac**, **13Ac** and **14Ac** (CDCl_3)

No	8Ac	9Ac	13Ac	14Ac
1	36.0 CH_2	36.0 CH_2	35.7 CH_2	35.7 CH_2
2	27.1 CH_2	27.1 CH_2	26.4 CH_2	26.3 CH_2
3	90.4 CH	90.2 CH	90.1 CH	90.1 CH
4	42.4 C	42.4 C	42.3 C	42.3 C
5	51.9 CH	51.9 CH	51.7 CH	51.7 CH
6	19.7 CH_2	19.7 CH_2	19.6 CH_2	19.6 CH_2
7	26.5 CH_2	26.5 CH_2	28.3 CH_2	28.3 CH_2
8	134.1 C	134.2 C	133.4 C	133.3 C
9	135.6 C	135.6 C	135.7 C	135.8 C
10	37.0 C	37.0 C	37.6 C	37.6 C
11	20.7 CH_2	20.7 CH_2	20.3 CH_2	20.3 CH_2
12	24.8 CH_2	24.8 CH_2	23.5 CH_2	23.5 CH_2
13	48.6 C	48.6 C	51.7 C	57.2 C
14	50.7 C	50.6 C	57.2 C	51.2 C
15	31.8 CH_2	31.8 CH_2	209.4 C	209.4 C
16	37.4 CH_2	37.4 CH_2	123.6 CH	123.6 CH
17	98.8 C	98.8 C	184.3 CH	184.4 CH
18	18.9 CH_3	18.9 CH_3	29.7 CH_3	29.0 CH_3
19	19.7 CH_3	19.7 CH_3	18.7 CH_3	18.7 CH_3
20	44.0 CH	44.0 CH	30.3 CH	30.4 CH
21	18.9 CH_3	18.9 CH_3	21.3 CH_3	21.1 CH_3
22	45.1 CH_2	45.1 CH_2	35.8 CH_2	35.8 CH_2
23	113.7 C	113.7 C	76.6 CH	76.6 CH
24	45.2 CH_2	45.2 CH_2	207.6 C	207.6 C
25	35.9 CH	35.9 CH	32.1 CH_2	32.1 CH_2
26	15.2 CH_3	15.2 CH_3	7.4 CH_3	7.4 CH_3
27	179.6 C	179.6 C	—	—
28	22.6 CH_3	22.6 CH_3	22.7 CH_3	22.7 CH_3
29	65.73 CH_2	65.7 CH_2	65.8 CH_2	65.8 CH_2
30	26.0 CH_3	26.0 CH_3	29.4 CH_3	29.4 CH_3
Glu 1'	103.2 CH	102.9 CH	103.9 CH	102.9 CH
2'	71.9 CH	71.9 CH	71.8 CH	71.9 CH
3'	73.3 CH	73.2 CH	73.2 CH	73.1 CH
4'	68.8 CH	69.2 CH	68.8 CH	69.0 CH
5'	71.8 CH	73.6 CH	71.9 CH	73.3 CH
6'	62.3 CH_2	67.6 CH_2	62.3 CH_2	67.7 CH_2
Arab 1''	—	100.4 CH	—	100.5 CH
2''	—	69.2 CH	—	69.2 CH
3''	—	70.3 CH	—	70.3 CH
4''	—	67.5 CH	—	67.5 CH
5''	—	62.9 CH_2	—	63.0 CH_2
OAc	20.8, 20.8, 20.8, 21.3, 21.9 CH_3	20.6, 21.2, 21.3, 20.9, 20.9, 21.1, 20.9	20.8, 21.0, 20.8, 20.8, 20.8, 21.3	20.8, 21.2, 21.3, 20.9, 20.9, 21.1, 20.9, 20.4
OAc	170.6, 169.4, 171.2, 169.6, 170.9 C	170.5, 169.7, 170.3, 170.6, 169.5, 169.4, 171.3	169.4, 169.6, 170.4, 170.7, 170.8, 171.2	170.5, 169.7, 170.3, 170.6, 169.5, 169.4, 171.3, 170.3

1692 ($\text{C}=\text{O}$ stretch); HRESIMS m/z 497.3225 $[\text{M} + \text{Na}]^+$ (calc. for $\text{C}_{29}\text{H}_{46}\text{O}_5\text{Na}$ 497.3243). NMR data are given in Tables 1 and 2.

3 β ,23S,29-Trihydroxy-27-norlanost-8,16-diene-15,24-dione (5). Colourless oil; $[\alpha]_{\text{D}}^{23.6} = +79.8$ (c 0.0020, CHCl_3); IR ν_{max} (NaCl) cm^{-1} 3433 ($\text{O}-\text{H}$ stretch) 3402 ($\text{O}-\text{H}$ stretch) and 1736 ($\text{C}=\text{O}$ stretch); HRESIMS m/z 495.3071 $[\text{M} + \text{Na}]^+$ (calc. for $\text{C}_{29}\text{H}_{44}\text{O}_5\text{Na}$ 495.3086). NMR data are given in Tables 1 and 2.

23S,29-Diacetoxy-3 β -[2',3',4',6'-tetra-*O*-acetyl-*O*- β -D-glucopyranoside]-27-norlanosta-8,16-diene-15,24-dione (6Ac). Clear gum; $[\alpha]_{\text{D}}^{23.6} = +12.2$ (c = 0.0007 g ml^{-1} , CHCl_3); IR ν_{max} (NaCl) cm^{-1} 2925, 2878 ($\text{C}-\text{H}$ stretch), 1240 cm^{-1} ($\text{C}-\text{O}$ stretch) and 1753 cm^{-1}

($\text{C}=\text{O}$ stretch); HRESIMS m/z 909.4254 $[\text{M} + \text{Na}]^+$ (calc. for $\text{C}_{47}\text{H}_{66}\text{O}_{16}\text{Na}$ 909.4243, 909.4248). NMR data are given in Tables 3 and 4.

(17S,23S)-29-Acetoxy-23,17-epoxy-3 β -[2',3',4'-tri-*O*-acetyl-*O*- β -D-glucopyranoside-(1'' \rightarrow 6')-2'',3'',4''-tri-*O*-acetyl-*O*- β -D-arabinopyranosyl]-lanost-8-en-23,27-olide (7Ac). Clear gum; $[\alpha]_{\text{D}}^{23.6} = +11.3$ (c = 0.0007 g ml^{-1} , CHCl_3); IR ν_{max} (NaCl) cm^{-1} 2925, 2878 ($\text{C}-\text{H}$ stretch), 1221 and 1248 cm^{-1} ($\text{C}-\text{O}$ stretch) and 1753 cm^{-1} ($\text{C}=\text{O}$ stretch). HRESIMS m/z 1125.4865 $[\text{M} + \text{Na}]^+$ (calc. for $\text{C}_{56}\text{H}_{78}\text{O}_{22}\text{Na}$ 1125.4882). NMR data are given in Tables 3 and 4.



(17S,23S)-29-Acetoxy-23,17-epoxy-3 β -[2',3',4'6'-tetra-O-acetyl-O- β -D-glucopyranoside]-lanost-8-en-23,27-olide (8Ac). Clear gum; $[\alpha]_D^{23.6} = +10.5$ ($c = 0.0012$ g ml $^{-1}$, CHCl $_3$); IR ν_{\max} (NaCl) cm $^{-1}$ 2922, 2847 (C–H stretch), 1225 cm $^{-1}$ (C–O stretch) and 1755 cm $^{-1}$ (C=O stretch). HRESIMS: $[M]^+$ not observed. NMR data are given in Tables 3 and 5.

(17S,23S)-29-Acetoxy-23,17-epoxy-3 β -[2',3',4'-tri-O-acetyl-O- β -D-glucopyranoside-(1'' \rightarrow 6')-2'',3'',4''-tri-O-acetyl-O- β -L-arabinopyranosyl]-lanost-8-en-23,27-olide (9Ac). Clear gum; $[\alpha]_D^{23.6} = +11.3$ ($c = 0.0007$ g ml $^{-1}$, CHCl $_3$); IR ν_{\max} (NaCl) cm $^{-1}$ 2925, 2878 (C–H stretch), 1221 and 1248 cm $^{-1}$ (C–O stretch) and 1753 cm $^{-1}$ (C=O stretch). HRESIMS m/z 1097.4880 $[M + Na]^+$ (calc. for C $_{55}$ H $_{78}$ O $_{21}$ Na 1097.4933). NMR data are given in Tables 3 and 5.

(17S,23S)-29-Acetoxy-23,17-epoxy-3 β -[2',3',4'6'-tetra-O-acetyl-O- β -D-glucopyranoside]-27-norlanost-8-en-24-one (10Ac). Clear gum; $[\alpha]_D^{23.6} = +12.4$ ($c = 0.0011$ g ml $^{-1}$, CHCl $_3$); IR ν_{\max} (NaCl) cm $^{-1}$ 2922, 2857 cm $^{-1}$ (C–H stretches), 1229 cm $^{-1}$ (C–O stretch) and 1744 cm $^{-1}$ (C=O stretch); HRESIMS m/z 830.4421 $[M]^+$ (calc. for C $_{45}$ H $_{66}$ O $_{14}$ 830.4453). NMR data are given in Tables 3 and 4.

(17S,23S)-29-Acetoxy-23,17-epoxy-3 β -[2',3',4'-tri-O-acetyl-O- β -D-glucopyranoside-(1'' \rightarrow 6')-2'',3'',4''-tri-O-acetyl-O- β -L-arabinopyranosyl]-27-norlanost-8-en-24-one (11Ac). Clear gum; $[\alpha]_D^{23.6} = +19.2$ ($c = 0.0005$ g ml $^{-1}$, CHCl $_3$); IR ν_{\max} (NaCl) cm $^{-1}$ 2937, 2868 cm $^{-1}$ (C–H stretches), 1223 cm $^{-1}$ (C–O stretch) and 1749 cm $^{-1}$ (C=O stretch); HRESIMS $[M]^+$ not observed. Base peak at m/z 587.1579, $[C_{23}H_{31}O_{16}Na + H]^+$ was observed. NMR data are provided in Tables 3 and 4.

(17S,23S)-29-Acetoxy-23,17-epoxy-3 β -[2',3',4'6'-tetra-O-acetyl-O- β -D-glucopyranoside]-27-norlanost-8-ene-15,24-dione (12Ac). Clear gum; $[\alpha]_D^{23.6} = +22.9$ ($c = 0.0016$ g ml $^{-1}$, CHCl $_3$); IR ν_{\max} (NaCl) cm $^{-1}$ 2921, 2847 cm $^{-1}$ (C–H stretches), 1222 cm $^{-1}$ (C–O stretch) and 1748 cm $^{-1}$ (C=O stretch); HRESIMS m/z 867.4147 $[M + Na]^+$ (calc. for C $_{45}$ H $_{64}$ O $_{15}$ Na 867.4143). NMR data are given in Tables 3 and 4.

(17S,23S)-29-Acetoxy-23,17-epoxy-3 β -[2',3',4'-tri-O-acetyl-O- β -D-glucopyranoside-(1'' \rightarrow 6')-2'',3'',4''-tri-O-acetyl-O- β -L-arabinopyranosyl]-27-norlanost-8-ene-15,24-dione (13Ac). Clear gum; $[\alpha]_D^{23.6} = +25.4$ ($c = 0.0011$ g ml $^{-1}$, CHCl $_3$); IR ν_{\max} (NaCl) cm $^{-1}$ 2922, 2848 cm $^{-1}$ (C–H stretches), 1225 cm $^{-1}$ (C–O stretch) and 1755 cm $^{-1}$ (C=O stretch); HRESIMS m/z 1083.4739 $[M + Na]^+$ (calc. for C $_{54}$ H $_{76}$ O $_{21}$ Na 1083.4776). NMR data are provided in Tables 3 and 5.

(17S,23S)-29-Acetoxy-23,17-epoxy-3 β -[2',3',4'-tri-O-acetyl-O- β -D-glucopyranoside-(1'' \rightarrow 6')-2'',3'',4''-tri-O-acetyl-O- β -L-arabinopyranosyl-(1''' \rightarrow 3')-2''',3'''-tri-O-acetyl-O- β -D-xylopyranosyl]-27-norlanost-8-ene-15,24-dione (14Ac). Clear gum; $[\alpha]_D^{23.6} = +24.5$ ($c = 0.0007$ g ml $^{-1}$, CHCl $_3$); IR ν_{\max} (NaCl) cm $^{-1}$ 2937, 2868 (C–H stretch) and 1223 cm $^{-1}$ (C–O stretch) and 1749 cm $^{-1}$ (C=O stretch). HRESIMS $[M]^+$ not observed. Base peak at m/z 803.2235 $[C_{32}H_{43}O_{22}Na + H]$ requires 803.2221 was observed. NMR data are provided in Tables 3 and 5.

Acknowledgements

This work was supported by the EPSRC Research Council [grant number EP/P50404X] and the PMI2 Project funded by the UK

Department for Business, Innovation and Skills (BIS) for the benefit of the United States Higher Education Sector and the UK Higher Education Sector (grant NPF204, awarded to Malcolm von Schantz). The views expressed are not necessarily those of BIS, nor British Council. This grant funded a visit by ML to the David H. Murdock Research Institute, Kannapolis, USA to record NMR spectra on the 950 MHz NMR instrument and we thank Dr Kevin Knagge for his assistance. Financial support by CNRS, Conseil Regional Champagne Ardenne, Conseil General de la Marne, Ministry of Higher Education and Research (MESR) and EU-programme FEDER to the PLAnET CPER project is gratefully acknowledged. We thank Mr Colin Sparrow of the Mass Spectrometry Research Facility, Department of Chemistry, University of Oxford for MS analysis. The authors wish to acknowledge The Developmental Therapeutics Program (DTP) of the National Cancer Institute of the United States (U.S.A) for performing the screening of compounds.

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