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Oleanane-type triterpenes of *Embelia schimperi* leaves

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Abstract

An investigation of an ethyl acetate extract of *Embelia schimperi* leaves has led to the isolation of 10 oleanane-type triterpenes characterized as 3β,16α-di-*O*-acetyl-13β, 28-epoxyoleanane (**1**), 3β-acetyl-16-oxo-13β, 28-epoxyoleanane (**2**), 3β-acetyl-16α-hydroxy-13β, 28-epoxyoleanane (**3**), 3β-acetyl-16α-hydroxyoleanane-13β, 28-olide (**4**), 3β-acetyl-28-hydroxy-16-oxo-12-oleanene (**5**), 3β, 28-di-*O*-acetyl-16α-hydroxy-12-oleanene (**6**), 3β-acetyl-11α, 28-dihydroxy-16-oxo-12-oleanene (**7**), 3β, 11α, 16α, 28-tetrahydroxy-12-oleanene (**8**), 3β-acetyl-16α, 28α-dihydroxy-13β, 28-oxydooleanane (**9**) and 3β, 28α-dihydroxy-16-oxo-13β, 28-oxydooleanane (**10**). The known compounds isolated from the same extract included 3β, 16α-dihydroxy-13β, 28-epoxyoleanane (protoprimulagenin A) (**11**), 3β-hydroxy-16-oxo-13β, 28-epoxyoxyoleanane (aegicerin) (**12**), 3, 16-dioxo-13β, 28-epoxyoleanane (embilionone) (**13**), 3β, 28-dihydroxy-16-oxo-12-oleanene (schimperinone) (**14**), taraxerone (**15**), taraxerol (**16**) and stigmasterol (**17**). Structure elucidations were carried out spectroscopically.
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Keywords: *Embelia schimperi*; Myrsinaceae; Oleanane tritrpenes; Leaves

1. Introduction

Embelia schimperi Vatke (Myrsinaceae) is a medicinal herb widely used as a remedy for antibacterial, antifungal and antihelmintic agents in Kenya (Kokwaro, 1976). Previous chemical analysis of the plant parts resulted into the isolation of benzoquinones and anthraquinones (Midiwo et al., 1988; Midiwo and Manguro, 1993), flavonol glycosides (Williams and Manguro, 1997) and oleanane-type triterpenes (Bittner et al., 2003). Recently, two new flavonol glycosides have been reported from this source (Manguro et al., 2004). In continuation with the phytochemical work on the plant, we herein report the isolation and structural elucidation of 10 new pentacyclic oleanane-type triterpenes (**1–10**) from the plant EtOAc extract. Known compounds isolated from the same extract included protoprimulagenin A (**11**), aegicerin (**12**), embilionone (**13**) and schimperinone (**14**) (Bittner et al., 2003) together with taraxerone (**15**), taraxerol (**16**) and stigmasterol (**17**) (Manguro et al., 1997; Sukurai et al., 1987). The present paper outlines the isolation and structural elucidation of new triterpenes.

2. Results and discussion

Compound **1**, obtained as colourless needles showed a significant absorption peak at 1734 (ester) cm^{-1} in the IR spectrum. Its ^1H NMR spectrum revealed the presence of seven tertiary methyls (δ 1.24, 1.04, 0.97, 0.96, 0.91, 0.89 and 0.86) and signals for two acetyl groups (δ 2.06 and 2.01). In addition, there were other significant peaks at δ 3.86 and 3.50 (each 1H, d, AB system, $J = 9.2$ Hz, suggesting the presence of a 13β, 28-epoxide), δ 4.90 (1H, dd, $J = 11.0$, 5.2 Hz, H-3) and 4.40 (1H, d, $J = 5.6$ Hz, H-16). These results and the ^{13}C NMR data (Table 1) were similar to those reported for protoprimulagenin A (**11**) (Bittner et al., 2003), except for the presence of two acetyl groups in **1**, confirmed by HRMS molecular ion peak at m/z 542.3971 ($C_{34}H_{54}O_5$). The positions of the acetyl groups,

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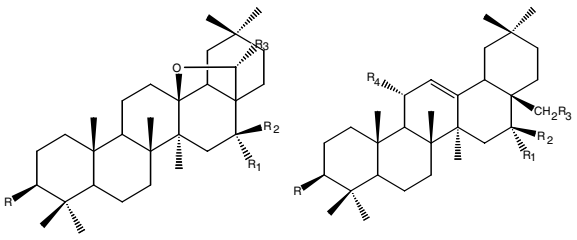

1 R = R_1 = OAc, R_2 = R_3= H

2 R = OAc, R_1 = R_2 = O, R_3 = H

3 R = OAc, R_1 = OH, R_2 = R_3 = H

4 R = OAc, R_1 = OH, R_2 = H, R_3 =O

9 R = OAc, R_1 = R_3 = OH, R_2 = H

10 R = R_3 = OH, R_1 = R_2 =O

11 R = R_1 = OH, R_2 = R_3 = H

12 R = OH, R_1 = R_2 = O, R_3 = H

13 R = R_1 = R_2 = O, R_3 =H

14 R = R_3 = OH, R_1 = R_2 = O, R_4 = H

5 R = OAc, R_1 = R_2 = O, R_3 = OH, R_4 =H

6 R = R_3 = OAc, R_1 = OH, R_2 = R_4 = H

7 R = OAc, R_1 = R_2 = O, R_3 = R_4 = OH

8 R = R_1 = R_3 = R_4 = OH, R_2 = H

the epoxide and the dispositions of H-3 and H-16 as β and α, respectively, were verified by a series of 2D NMR experiments, whereby the key proton resonances were assigned by HMQC correlations. The long-range correlation (HMBC) cross-peaks were observed between H-28 (δ 3.86) and C-16 (δ 78.4); between H-16 (δ 4.40) and C-28 (δ 76.1); between H-16 and C-14 (δ 49.4); between H-15 (δ 1.84) and C-13 (δ 86.0), and between H-3 (δ 4.90) and C-5 (δ 55.4). These results together with information from the CIMS data evidenced a protoprimulagenin A derivative with acetyl groups at C-3 and C-16, and an epoxide linkage originating through hydroxylation at C-28 generating a hemiacetal function (Alliota et al., 1992; Isao et al., 1980; Kitagawa et al., 1977; Calis, 1987), a fact further supported by NOESY cross-peaks between H-28 and H-19 (δ 1.62), and between H-28 and Me-29 (δ 0.91), which is consistent with the *R*-configuration of C-28 (Sindambiwe et al., 1998; Aperas et al., 1999). Based on these results, compound **1** was concluded to be 3β, 16α-di-*O*-acetyl-13β, 28-epoxyoleanane.

Compound **2**, analysed for $C_{32}H_{50}O_4$ (CIMS, ^{13}C NMR and DEPT). Data from ^1H and ^{13}C NMR indicated a secondary metabolite with structure closely similar to that of 3β-hydroxy-16-oxo-13β, 28-epoxyoleanane (aegicerin) (**12**) (Bittner et al., 2003) with major structural difference being the acetyl group in the former. The CIMS gave daughter ions at *m*/*z* 249 ($C_{16}H_{25}O_2$) and 248 ($C_{16}H_{24}O_2$) previously observed in **12** formed by cleavage of the 9–11 and 8–14 bonds, thus suggesting the presence of a 13β, 28-epoxide and a 16-ketonic carbonyl group (Bittner et al., 2003; Isao et al., 1972). This was further corroborated by the HMBC spectrum, whereby the proton signal at δ 1.94 (H-18) correlated with C-16 (δ 213.1) and C-28 (77.5). On the other hand, in the ^1H–^1H homonuclear chemical correlation spectrum of 2, the comparatively low field oxymethine proton at δ 4.55 showed cross-peaks with other two protons and hence was assigned to C-3, further confirmed by HMBC as was evidenced by cross-peaks between the H-3 (δ 4.55) and C-5 (δ 57.8). The large coupling constant ($J = 11.6$ and 5.2 Hz) allowed the assignment of β and equatorial orientation to the acetyl group. The spin decoupling combined with NOESY experiments allowed complete assignment of the stereochemistry of compound 2. Thus, the structure of **2** was concluded to be 3β-acetyl-16-oxo-13β, 28-epoxyoleanane.

Compound **3** afforded a HRMS molecular ion peak at *m*/*z* 500.3866 corresponding to the formula $C_{32}H_{52}O_4$, which was further supported by a combined application of ^1H, ^{13}C NMR, DEPT and IR data. The ^1H and ^{13}C NMR data were similar to those recorded for compound **2** except for the signal attributed to a carbinol methine proton at δ 3.94 assigned to H-16 in **3**. The long-range H,C correlation (HMBC, $J_{2,3} = 8$ Hz) spectrum exhibited correlations between H-28 (δ 3.76 and C-16 (δ 76.8); between H-16 (δ 3.94) and C-14 (44.0); H-18 (δ 1.74) and C-16 confirming the 13, 28-epoxy linkage. A NOESY experiment confirmed the foregoing evidence by showing cross-peaks between H-28, Me-29 and H-15, which are consistent with the *R*-configuration at C-28 (Calis, 1987; Alliota et al., 1992). From the spectroscopic data as well as comparison with the data from known compounds, **3** was identified as 3β-acetyl-16α-hydroxy-13β, 28-epoxyoleanane.

Compound (**4**) showed the presence of hydroxyl (3450 cm^{-1}), lactone (1760 cm^{-1}) and ester (1730 cm^{-1}) absorption bands in the IR spectrum. The ^1H NMR spectrum exhibited two oxymethine protons [4.50 (1H, dd, $J = 11.0$, 5.0 Hz) and 3.90 (1H, d, $J = 5.4$ Hz)], an acetyl group δ 2.01 (s, 3H) and seven tertiary methyls (δ 1.18, 1.05, 0.96, 0.92, 0.88, 0.86, 0.81). The ^{13}C NMR spectrum further supported both the ^1H NMR and IR by exhibiting a lactone carbon at δ 180.1 and two methine carbons at δ 80.0 and 73.4 considered to be attached to oxygen functionalities. These together with an acetyl group, seven methyls, ten methylenes, five methines and eight quaternary carbons (from DEPT) agreed with the molecular formula $C_{32}H_{50}O_5$, a fact supported by the HRMS molecular ion peak at *m*/*z* 514.3658. Based on the above data, the C-skeleton containing 32 carbons, including an acetyl group and seven tertiary methyl groups inferred that **4** is an oleanane-type triterpene (De Lampasona et al., 1998; Mahto et al., 1992). The presence of quaternary carbon signals at δ 96.4 (C-13) and 180.1 (C-28) in the ^{13}C NMR along with prominent peak at *m*/*z* 264 $[C_{16}H_{24}O_3]^+$ in the CIMS supported the lactone moiety to be between C-13 and C-28 (Siddiqui et al., 2000). On the other hand, the NMR spectrum signals at δ_H 4.50 (δ_c 80.0 in DEPT and HMQC) and δ_H 3.90 (δ_c 73.4 in DEPT and HMQC) were ascribable to 3β and 16α-protons, respectively and confirmed by HMBC correlations as observed between H-5 (δ 0.76) and C-3 (δ 80.0); between H-18 (δ 1.46) and C-16 (δ 73.4). The relatively downfield δ 4.50 inferred that the acetyl group was

Table 1
^{13}C NMR of compounds **1–10**

Carbon	1	2	3	4	5	6	7	8	9	10
1	39.5	39.6	38.9	38.9	39.4	39.0	39.5	38.9	38.7	39.5
2	28.9	26.6	27.4	28.4	27.2	28.4	28.0	27.6	27.5	27.6
3	80.3	81.0	80.4	80.0	79.6	79.9	78.9	76.3	80.0	74.4
4	40.5	42.3	39.3	39.4	39.7	38.8	39.3	39.0	39.1	39.0
5	55.4	57.8	56.6	56.0	55.6	56.0	56.0	55.8	54.9	55.7
6	18.1	18.4	18.2	18.6	18.0	17.9	18.3	18.7	18.0	18.2
7	32.2	33.2	32.7	33.4	32.2	32.4	33.0	33.3	33.8	32.9
8	42.4	41.6	42.0	42.6	40.7	41.0	40.5	41.0	43.1	42.0
9	50.2	40.4	51.4	49.9	48.3	48.6	49.1	47.8	50.3	50.3
10	37.1	36.5	37.0	37.0	37.4	36.9	36.7	37.1	32.9	36.9
11	19.4	19.5	20.1	20.1	23.8	24.4	70.5	71.8	19.3	18.6
12	33.0	32.6	34.7	34.2	122.4	123.4	121.7	123.0	33.4	33.6
13	86.0	85.9	86.4	96.4	145.6	146.3	146.0	145.6	87.2	86.3
14	49.4	47.0	44.0	44.1	46.8	42.8	43.3	42.3	44.1	44.0
15	44.8	44.4	35.9	36.2	44.3	35.1	36.6	36.0	35.9	35.4
16	78.4	213.1	76.8	73.4	212.2	75.4	212.4	72.8	69.4	212.7
17	57.0	55.0	43.6	46.5	54.9	44.1	57.8	41.5	53.0	53.3
18	52.6	53.4	51.2	51.3	47.9	42.3	51.9	46.3	46.6	46.0
19	40.0	39.6	39.1	40.0	47.2	48.4	48.5	45.6	37.9	38.8
20	32.2	32.0	31.7	32.1	31.9	31.7	30.8	32.0	36.9	30.9
21	35.6	36.0	36.8	37.3	36.7	37.6	36.0	36.8	37.2	37.4
22	34.8	33.8	32.7	33.1	29.4	30.8	29.9	31.2	33.6	34.0
23	27.4	27.2	28.4	28.4	28.1	28.0	27.4	28.2	28.2	27.6
24	16.7	20.1	16.6	16.6	17.0	16.4	16.4	16.0	16.2	15.9
25	15.8	16.2	16.0	15.8	16.4	16.1	15.9	15.7	16.5	15.7
26	19.0	18.9	18.5	18.6	18.3	18.6	17.8	17.8	18.7	17.7
27	22.4	22.1	19.5	19.9	27.1	27.8	27.0	26.9	19.1	18.8
28	76.1	77.5	78.2	180.1	62.0	76.5	63.0	61.5	99.6	100.4
29	33.2	34.0	33.6	33.4	34.0	34.1	33.1	33.8	32.8	31.3
30	23.7	24.2	23.6	25.0	25.4	25.2	25.8	25.3	24.5	25.3
Ac	171.0	169.9	170.4		171.4	168.2	170.0		170.2	
	170.4	23.5	24.3		25.6	170.8	23.6		23.6	
	25.6					23.6				
	24.7					24.5				

at this position. The disposition of an acetyl group as β and the hydroxyl as α was confirmed by cross-peaks between CH_3-24 (δ 0.81) and acetate (δ 2.01), and between H-15 (δ 2.05), H-22 (δ 1.25) and H-16 (δ 3.90) in the NOESY plot. Thus, compound **4** was concluded to be 3β-acetyl-16α-hydroxyoleanane-13β, 28-olide.

Compound **(5)**, $C_{32}H_{50}O_4$ (^{13}C NMR, DEPT and HRMS), showed the presence of hydroxyl (3450 cm^{-1}), acetyl group (1732 cm^{-1}), ketone (1700 cm^{-1}) and a tri-substituted double bond (1647 cm^{-1}) in the IR spectrum. The broadband decoupled ^{13}C and DEPT NMR spectra of compound **5** afforded 32 signals accounted for by 8 methyl, 10 methylene, 5 methine and 9 quaternary carbons. The ^1H and ^{13}C NMR data of **5** resembled closely those of 3β, 28-dihydroxy-16-oxo-12-oleanene (**14**) (Bittner et al., 2003) except for the acetyl group substitution in ring A at C-3 as evidenced by characteristic peak at δ 4.70 (1H, t, $J = 10.5$, 5.1 Hz) with corresponding ^{13}C NMR peak at δ 79.6. Unequivocal information on the ring system and substitution pattern in **5** was obtained from CIMS spectrum, whereby two characteristic peaks at m/z 249 $[C_{16}H_{25}O_2]^+$ (100%) and 248 $[C_{16}H_{24}O_2]^+$ (70%) indicated the retro-Diels-Alder cleavage commonly found in the spectra of olean-12-ene or urs-12-ene derivatives possessing acetyl group in rings A/B and a hydroxyl and a ketone in rings D/E (Bittner et al., 2003; Ohtani et al., 1993; Napoli et al., 1992; Isao et al., 1974). The olean-12-ene was inferred from the chemical shifts of C-12 (δ122.4) and C-13 (δ145.6) (Hiradate et al., 1999), a fact corroborated by the presence of a peak at δ 5.30 (d, $J = 3.4$ Hz) and seven tertiary methyl groups (δ 1.25, 0.97, 0.96, 0.89, 0.87, 0.82 and 0.80) in the NMR spectrum. In the HMBC spectrum, the proton signal at δ 2.15 (H-18) was correlated with the carbon signals at δ 62.0 (C-28). Based on the above information, **5** was identified as 3β-acetyl-28-hydroxy-16-oxo-12-oleanene.

Compound **6**, molecular formula, $C_{34}H_{54}O_5$ (HRMS; m/z 542.3971), showed seven tertiary methyls (δ 1.20, 1.05, 0.96, 0.94, 0.92, 0.90, 0.86, each 3 H), an olefinic proton (δ 5.16, t, $J = 3.6$ Hz, H-12), two oxymethine protons (δ 4.65, t, $J = 12.0$, 5.1 Hz, H-3 and δ 3.94, d, $J = 5.5$ Hz, H-16), oxymethylene protons (δ 4.30, dd, $J = 11.4$, 5.3 Hz and 3.76, dd, $J = 11.4$, 3.7 Hz, CH_2-28) and two acetyl groups (δ 2.03 and 2.01, s, each 3H) in the ^1H NMR spectra. The ^{13}C NMR spectrum exhibited 34 carbons ascribable to 10 methylenes, 9 methyls, 6 methines and 9 quaternary carbons by DEPT spectrum. Both the ^1H and ^{13}C NMR spectra depicted data typical of a triterpene of

either oleanane or ursane skeleton containing a double bond between C-12 and C-13 and two acetyl groups (Bittner et al., 2003; Sukumar et al., 1995). This was further supported by the characteristic retro–Diels–Alder cleavage of Δ^{12}-pentacyclic triterpene skeleton leading to *m/z* 292 $[C_{18}H_{28}O_3]^+$ and 249 $[C_{16}H_{25}O_2]^+$, thus indicating that one acetyl group was present in the rings A/B while the other acetyl group and a hydroxyl were in rings D/E of the molecule (Hiradate et al., 1999; Sukumar et al., 1995). In fact, the relatively low field values for the oxymethine (δ 4.65) and oxymethylene protons (δ 4.30 and 3.76) in comparison with primulagenin A (Bittner et al., 2003; Calis et al., 1992; Sindambiwe et al., 1996), indicated that the acetyl groups were at C-3 and C-28, confirmed by HMBC experiments (Fig. 1) and further supported by the CIMS splitting pattern (Fig. 2). In the ^{13}C NMR spectrum, the olefinic carbons C-12 and C-13 appeared at δ 123.4 and 146.3, respectively. The low field value of C-13 suggested that **6** is an oleanane derivative rather than an ursane (Bittner et al., 2003; Napoli et al., 1992). The location and the α-disposition of the secondary hydroxyl group at C-16 was established by both HMBC and NOESY correlations (Fig. 1). Thus, the structure **6** was therefore concluded to be 3β, 28-diacetyl-16α-hydroxy-12-oleanene.

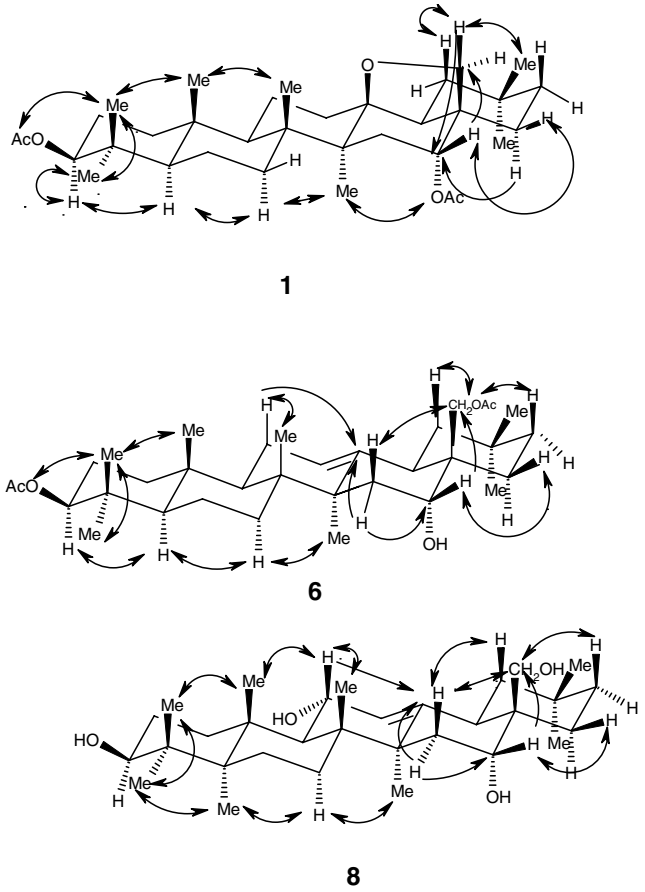

Fig. 1. Significant HMBC and NOESY correlations for compounds **1**, **6** and **8**.

Compound (**7**), $C_{32}H_{50}O_5$, possessed hydroxyl, ester, ketone and a double bond from the IR spectrum (3530, 1734, 1703, 1640 cm^{-1}). Its ^{1}H NMR spectrum showed a vinyl proton (δ 5.15, t, $J = 4.1$ Hz), allylic methine proton attached to an oxygen functionality (δ 3.93, $J = 3.5$ Hz), an oxymethine proton (δ 4.90, t, $J = 11.6$, 5.4 Hz), a terminal hydroxymethylene (δ 4.20, dd, $J = 11.2$, 5.6 Hz, and δ 3.95, dd, $J = 11.2$, 3.6 Hz), an acetyl group (δ 2.07, s) and seven tertiary methyls (δ 1.21, 1.05, 1.00, 0.97, 0.94, 0.92, 0.84, all s, each 3 H). The ^{13}C NMR spectrum was similar to that of 3β-acetyl-28-hydroxy-16-oxo-olean-12-ene (3β-acetylschimperinone) (**5**), confirmed from the assignment of data aided by examination of 2D NMR. Thus, it was revealed that the difference between compounds **5** and **7** was allylic hydroxyl group at C-11 in the latter, which was confirmed by HMBC correlation peaks between H-11 (δ 3.93) and carbons C-12 (δ 121.7), C-13 (δ 146.0), C-8 (40.5), C-9 (49.1) and C-10 (δ 36.7) and further corroborated by the CIMS peaks at *m/z* 249 $[C_{16}H_{25}O_2]^+$ and 265 $[C_{16}H_{25}O_3]^+$. The α-disposition of the proton at C-11 was concluded from NOESY spectrum as evidenced by cross-peaks between C-29 methyl (δ 0.92), C-28 methylene (δ 4.20), H-15 (δ 2.73) and H-11 (δ 3.93). Thus, compound **7** was concluded to be 3β-acetyl-11α, 28-dihydroxy-16-oxo-12-oleanene (3β-acetyl-11α-dihydroxyschimperinone).

Compound (**8**), colourless crystals afforded HRMS molecular ion peak at *m/z* 474.3709 $[M]^+$, corresponding to the formula $C_{30}H_{50}O_4$. Both its ^{1}H and ^{13}C NMR spectra showed signals for seven tertiary methyls, three oxymethines, a terminal hydroxymethylene and a substituted olefinic bond, thus suggesting that the compound might be an olean-12-ene (Sukumar et al., 1995). Support for this was further provided by characteristic retro-Diels-Alder fragmentation of Δ^{12}-pentacyclic triterpene skeleton giving rise to peaks at *m/z* 207 ($C_{14}H_{21}O$) and 266 $[C_{16}H_{26}O_3]^+$, which together with fragments at *m/z* 189 $[C_{14}H_{21}]^+$ (resulting from *m/z* 207), 235 $[C_{15}H_{23}O_2]^+$ and 217 $[C_{15}H_{21}O]^+$ (resulting from *m/z* 266), indicate the presence of one hydroxyl group in rings A/B, two other hydroxyls and a terminal hydroxymethylene were in rings D/E part of the molecule (as evidenced by the peak at *m/z* 266 $[C_{16}H_{26}O_3]^+$) (Bittner et al., 2003). On biogenetic grounds, the hydroxyl group on rings A/B was at C-3 on the basis of its configuration as evidenced by the chemical shift value and coupling constants, and further confirmed by peaks at *m/z* 207 and 189 (Tanaka and Matsunaga, 1989). Similarly, the signal at 4.00 (dd, $J = 8.7$, 3.4 Hz) was typical of allylic oxymethine proton and was positioned at C-11. Its doublet of doublet nature is characteristic of an oxymethine proton next to two protons on adjacent carbons (Calis, 1989). The location of the third hydroxyl at C-28, respectively was discernible from further splitting of *m/z* 266 ($C_{16}H_{26}O_3$) (see experimental section) and confirmed by important HMBC correlation peaks observed between H-16 (δ 3.85) and C-28 (δ 61.5); between H-18 (δ 2.16)and C-28, and between H-16 and C-14 (δ 42.3). The configuration of the 11α and 16α-dihydroxy groups were

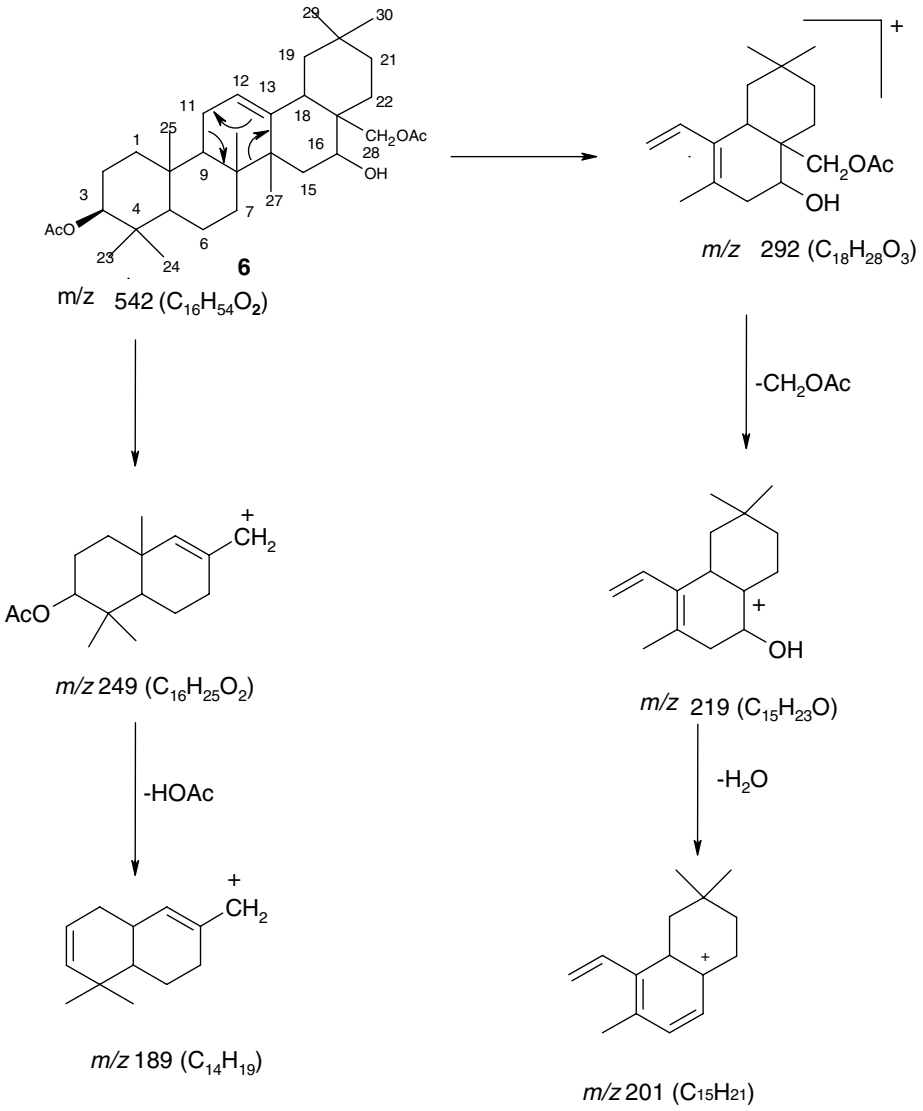

Fig. 2. Fragmentation of compound **6** in CIMS.

confirmed from NOESY experiments (Fig. 1). On this basis **8** was established to be 3β, 11α, 16α, 28-tetrahydroxy-12-oleanene (11α-hydroxyprimulagenin).

Compound **9** was colourless crystals with a molecular formula $C_{32}H_{54}O_5$ determined from HRMS (*m/z* 516.3815), ^{13} C and DEPT NMR data. Its ^{1}H NMR spectrum showed seven tertiary methyl protons (δ 1.25, 1.03, 0.98, 0.96, 0.91, 0.88 and 0.84, each s) and an acetyl group (δ 2.05, s). Additional significant signals observed at δ 4.70 (1H, dd, $J = 10.5$, 5.1 Hz, H-3), 4.12 (1H, d, $J = 3.5$ Hz, H-16) and a typical singlet at δ 4.80 (1H, s, H-28) suggested the presence of a 13, 28-oxydo moiety with a hemiacetal function at C-28 (Calis et al., 1992; Calis, 1989). The ^{13}C NMR further supported this: δ 99.6 (H–C(O–)–OH) and 87.2 (–C(O–)–). The spectrum also indicated the presence of two oxygen-bearing carbon methines showing resonances at δ 80.0 (C-3) and 69.4 (C-16). The CIMS strong fragments at *m/z* 249 and 264 were attributable to the fragments arising from the cleavage of the 9–11 and 8–14 bonds and their formation further supported the presence of a hemiacetal function and a hydroxyl group in rings D/E, whereas the acetyl group was present in rings A/B (Calis et al., 1992). The site of esterification as C-3 was evident from the long-range C–H correlations between C-3 (δ 80.0) and H-5 (δ 0.80) as observed in the HMBC spectrum. Similarly C-16 was observed to correlate with H-28 in the same experiment. Detailed analysis of 2D NMR (HMQC, ^{1}H–^{1}H COSY, HMBC and NOESY) spectra allowed complete ^{1}H and ^{13}C NMR data assignments and the unequivocal establishment of structure of **9** as 3β-acetyl-16α,28α-dihydroxy-13β, 28-oxydooleanane.

Compound **10** HRMS afforded a parent molecular ion peak at *m/z* 472.3553 compatible with a $C_{30}H_{48}O_4$ formula. Its decoupled ^{13}C NMR spectrum exhibited 30 distinct carbon signals ascribable to seven methyls, ten methylenes, five methines and eight non-protonated carbon atoms by

DEPT experiments, accounting for 46 of the 48 protons in the molecule. The remaining two protons were part of the hydroxyl functionalities as evidenced by ^{13}C NMR peaks 100.4 (C-28) and 74.4 (C-3). The NMR spectral data were closely related to those of **9** except that the ^{13}C NMR spectrum of **10** showed a downfield shift at δ 212.7 but lacked an acetyl group. This strongly suggested that in **10**, hydroxyl and ketonic groups have replaced acetyl and OH functionalities at C-3 and C-16, respectively. The foregoing evidence was supported by mass spectrum fragmentation pattern characteristic of saturated pentacyclic triterpenes, whereby peaks at *m/z* 207 and 265 signified cleavage of 9–11 and 8–14 bonds (Calis et al., 1992; Calis, 1989; Baigent et al., 1976). The formation of these fragments led to the conclusion that the hemiacetal and ketonic functionalities were in rings D/E while the hydroxyl was present in rings A/B. The positions of the hemiacetal, ketonic and hydroxyl groups were confirmed from HMBC and NOESY correlation, and supported by ^{1}H NMR spectral evidences; in particular, the HMBC correlations observed between H-28 (δ 5.28) and C-16 (δ 212.7); between H-18 (δ 2.20) and C-28 (δ 100.4), and between H-5 (δ 0.76)and C-3 (δ 74.4). Therefore on the basis of spectroscopic data as well as comparison with known compounds, compound **10** was structurally concluded to be 3β, 28α-dihydroxy-16-oxo-13β, 28-oxydooleanane.

3. Experimental part

3.1. General experimental procedure

The IR data were recorded on Perkin-Elmer FTIR 600 series, respectively. The NMR data were taken in $CDCl_3$ and $CDCl_3$-DMSO-d_6 on a Bruker NMR Advance Ultrashield TM spectrometer operating at 500 and 125 MHz. The CIMS data were obtained on a MAT 8200 A Varian Bremen instrument. Preparative HPLC was performed on JASCO Labor und Daten Technik Deutschland GmBH (RP-18, 250 × 20 mm, 7.4 μm JASCO Kromasil 100).

3.2. Plant material

The plant, *E. schimperi* parts namely; leaves and stem bark were collected from Ngong forest about 30 km south of Nairobi, Kenya in August 2001. Voucher specimens (No. 200/8 NMU) were identified after comparison with authentic samples at the Kenya National Museum.

3.3. Extraction and isolation

Air-dried powdered leaves (approx. 3 kg) were extracted sequentially with EtOAc (7.5 l, one week) and MeOH (7.5 l, one week). The extracts were separately filtered and concentrated to give dark green residues of 105 and 165 g, respectively. The MeOH extract was kept for future use while a portion of EtOAc extract (≈100 g) was chromatographed over silica gel (medium pressure, pressure ≅1 bar); eluted with *n*-hexane-EtOAc mixture with increasing concentration of the more polar solvent, EtOAc, CH_2Cl–MeOH mixture (9:1, 4:1, 7:3, and 2:1) and lastly elution was concluded with MeOH. A total of 273 fractions, each 20 ml were sampled and their homogeneity monitored by TLC; solvent systems (n-Hexane-EtOAc 9:1, 4:1, 7:3, 1:1, and CH_2Cl_2–MeOH, 19.1, 9:1, 4:1 and 2:1). The spot were visualised as violet after spraying with anisaldehyde followed by heating and those showing similar TLC profiles were combined to give five pools (I–V). Pool I (fractions 1–55, 17.6 g) was further subjected to repeated flash chromatography using eluent pentane-EtOAc (9:1) followed by the same solvent system (4:1) to afford stigmasterol (**17**; 80 mg), 3β,16α-di-*O*-acetyl-13β, 28-epoxyoleanane (**1**; 50 mg), taraxerone (**15**; 90 mg), 3β-acetyl-16-oxo-13β, 17-epoxyoleanane (**2**; 50 mg) and 3,16-dioxo-13β, 28-epoxyoleanane (embilionone) (**13;** 43 mg). Fractions 56–101 (Pool II, 15.5 g) was similarly subjected to repeated flash chromatography: eluent pentane-EtOAc (4:1) followed by the same solvent in the ratio 3:2, collecting 10 ml each to give 3β-acetyl-16α-hydroxy-13β, 28-epoxyoleanane (**3**; 85 mg), 3β-acetyl-16α-hydroxyoleanane-13β, 28-olide (**4;** 65 mg), 3β-acetyl-28-hydroxy-16-oxo-12-oleanene (**5**; 25 mg), 3β-28-dihydroxy-16-oxo-12-oleanene (**14**; 51 mg) and taraxerol (**16**; 105 mg).

Pool III (fractions 102–143, 8.7 g) upon further repeated purification as described above using pentane-EtOAc (3:2) followed by the same solvent in the ratio 7:3, collecting 10 ml each gave 3β-hydroxy-16-oxo-13β, 28-epoxyoleanane (**12**: 50 mg), and a further portion **3** (25 mg). Fractions 144–200 (Pool IV, 9.4 g) contained four components which were resolved using flash chromatography; eluent:CH_2Cl_2–MeOH (99:1, 19:1) into protoprimulagenin A (**11**; 55 mg), 3β, 28-di-*O*-acetyl-16α-hydroxy-12-oleanene (**6**; 34 mg), a further portion of **12** (23 mg) and 3β-acetyl-11α, 28-dihydroxy-16-oxo-12-oleanene (**7**; 37 mg). Pool V (fraction 201–273, 13 g) mainly from methanol elution was further subjected to medium pressure chromatography with CH_2Cl_2–MeOH mixture of increasing polarity of the latter to give 120 fractions of 20 ml each. The eluates were found to contain mixtures which were further purified by reverse phase HPLC using gradient elution of acetonitrile–MeOH (starting with 100% acetonitrile and finishing with 100% MeOH; mobile phase flow-rate, 12 ml/min and pressure of 6.1 mPa; time per run was 40 min), injecting 10 μl each time to give 3β, 11α,16α, 28-tetrahydroxy-12-oleanene (11α-hydroxyprimulagenin A) (**8**; 62 mg), 3β-acetyl-16α, 28α-dihydroxy-13β, 28-oxydooleanane (**9**; 53 mg) and 3β, 28α-dihydroxy-16-oxo-28-oxydooleanane (**10**; 45 mg), respectively.

3.4. 3β,16α-Di-O-acetyl-13β, 28-epoxyoleanane (*1*)

Colourless crystals (*n*-pentane-EtOAc), m.p. 241-243 °C. $[\alpha]_D^{25} = -6°$ ($c = 0.6$, CH_2Cl_2). IR ν_{max} (KBr) cm^{-1}: 2925, 2850, 1734 (ester), 1385, 1235, 1120, 1080,

1050, 890. ^{1}H NMR ($CDCl_3$,500 MHz) δ: 4.90 (1H, dd, $J = 11.0$, 5.2 Hz, H-3), 4.40 (1H, d, $J = 5.6$ Hz, H-16), 3.86 (1H, d, $J = 9.2$ Hz, H-28a), 3.50 (1H, d, $J = 9.2$ Hz, H-28b), 2.81 (1H, d, $J = 16.4$ Hz, H-15eq), 2.53 (1H, ddd, $J = 16.0$, 10.5, 7.5 Hz, H-2ax), 2.06 (3H, s, OAc), 2.01 (3H, s, OAc), 1.96 (1H, m, H-1), 1.93 (1H, m, H-7), 1.91 (1H, dd, $J = 11.6$, 4.6 Hz, H-18), 1.84 (1H, d, $J = 16.5$Hz, H-15), 1.77 (1H, ddd, $J = 18.3$, 14.2, 5.4 Hz, 11ax), 1.70-1.24 (7H, m, H-1, CH_2-2, H-12, CH_2-6, H-7), 1.62 (1H, m, H-19a), 1.20-1.00 (5H, m, H-21, H-9, H-12, H-19b, H-22), 1.24 (3H, s, Me-27), 1.04 (3H, s, Me-25), 0.97 (3H, s, Me-26), 0.96 (3H, s, Me-23), 0.91 (3H, s, Me-29), 0.89 (3H, s, Me-30), 0.86 (3H, s, Me-24), 0.57 (1H, m, H-5). ^{13}C NMR ($CDCl_3$,125 MHz) data, see Table 1. CIMS m/z (%): 542 $[M]^+$ (6), 527 $[M-Me]^+$ (3), 482 $[M-HOAc]^+$ (13), 422 $[M-2xHOAc]^+$ (24), 411 (4), 409 (12), 391 (13), 385 (12), 292 (35), 261 (7), 249 (18), 236 (21), 235 (46), 220 (57), 219 (40), 207 (100), 201 (17), 189 (34), 55 (70), (42 66). HRMS m/z: 542.3971 (calcd for $C_{34}H_{54}O_5$, 542.3893).

3.5. 3β-Acetyl-16-oxo-13β, 28-epoxyoleanane (2)

Colourless crystals (pentane-EtOAc), m.p. 220–222 °C. $[\alpha]_D^{25} = -34°$ (c = 1.0, CH_2Cl_2). IR ν_{max} (KBr) cm^{-1}: 2922, 2855, 1730 (ester), 1701 (C=O), 1450, 1380, 1215, 1120, 1080, 1030, 995, 780. ^{1}H NMR ($CDCl_3$,500 MHz) δ: 4.55 (1H, dd, $J = 11.6$, 5.2 Hz, H-3), 3.86 (1H, d, $J = 10.0$ Hz, H-28a), 3.67 (1H, d, $J = 10.0$ Hz, H-28b), 2.71 (1H, d, $J = 16.4$ Hz, H-15eq), 2.22 (1H, ddd, $J = 14.1$, 5.0, 2.6 Hz, H-22eq), 2.05 (3H, s, OAc), 1.94 (1H, dd, $J = 12.0$, 3.4 Hz, H-18), 1.88 (1 H, ddd, $J = 17.6$, 12.4, 3.8 Hz, H-7ax), 1.75 (1H, dt, $J = 14.0$, 4.6 Hz, H-1ax), 1.83 (1H, d, $J = 16.2$ Hz, H-15), 1.66 (1H, ddd, $J = 18.0$, 11.4, 4.2 Hz, H-11ax), 1.57–1.34 (9H, m, H-19eq, CH_2-2, H-12, CH_2-6, H-7, H-11, H-21), 1.31 (1H, t, $J = 14.3$ Hz, H-19ax), 1.24 (3H, s, Me-27), 1.20 (1H, m, H-21), 1.18 (3H, s, Me-25), 1.14 (2H, m, H-9 and H-22), 1.00 (3H, s, Me-26), 0.95 (3H, s, Me-23), 0.91 (3H, s, Me-30), 0.88 (1H, m, H-1), 0.86 3H, (s, Me-29), 0.84 (3H, s, Me-24), 0.76 (1H, dd, $J = 11.6$, 2.5 Hz, H-5). ^{13}C NMR ($CDCl_3$,125 MHz) data, see Table 1. CIMS (m/z (%)): 498 $[M]^+$ (10), 483 (5), 458 (15), 456, 438 $[M-HOAc]^+$ (14), 407 $[M-HOAc-CH_2OH]^+$ (11), 250 (5), 249 (20), 248 (50), 236 (8), 235 (10), 219 (8), 217 (25), 207 (44), 203 (20), 202 (40), 189 (40), 85 (45), 59 (31), 42 (100). HRMS m/z 498.3709 (calcd for $C_{32}H_{50}O_4$, 498.3631).

3.6. 3β-Acetyl-16α-hydroxy-13β, 28-epoxyoleanane (3)

The compound was isolated as colourless crystals from EtOAc–MeOH–Pentane mixture, m.p. 245–246 °C. $[\alpha]_D^{25} = -25°$ ($c = 0.60$, CH_2Cl_2). IR ν_{max} (KBr) cm^{-1}: 3350 (OH), 2930, 2860, 1732 (ester), 1365, 1300, 1140, 1097 (C–O–C), 980. ^{1}H NMR ($CDCl_3$,500 MHz) δ: 4.50 (1H, dd, $J = 10.7$, 5.4 Hz, H-3), 3.94 (1H, d, $J = 5.2$ Hz, H-16), 3.76 (1H, d, $J = 9.0$ Hz, H-28a), 3.43 (1H, d, $J = 9.0$ Hz, H-28b), 2.16 (1H, dd, $J = 14.4$, 11.8 Hz, H-19ax), 2.08 (1H, dd, $J = 14.8$, 5.0 Hz, H-15 eq), 2.03 (3H, s, OAc), 1.94 (1H, m, H-22ax), 1.79 (1H, m, H-15ax), 1.74 (1H, m, H-18), 1.39–1.76 (9H, m, CH_2-2, CH_2-6, CH_2-7, H-8, H-19eq, H-11), 1.24–1.35 (4H, m, H-11, H-12, CH_2-22), 1.23 (3H, s, Me-27), 1.10 (3H, s, Me-25), 0.98 (3H, s, Me-26), 0.95 (3H, s, Me-23), 0.90 (3H, s, Me-30), 0.89 (3H, s, Me-29), 0.85 (3H, s, Me-24), 1.00–1.30 (4H, m, CH_2-21, H-9, H-22), 0.88 (1H, m, H-1), 0.80 (1H, dd, $J = 12.2$, 1.8 Hz, H-5). ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Table 1. CIMS m/z (%): 500 $[M]^+$ (10), 482 $[M-H_2O]^+$ (10), 540 $[M-HOAc]^+$ (50), 457 (2), 441 (7), 426 (21), 422 $[M-H_2O-HOAc]^+$ (9), 249 (100), 248 (53), 236 (20), 220 (17), 219 (35), 207 (80), 189 (11). HRMS m/z 500.3866 (calcd for $C_{32}H_{52}O_4$, 500.3787).

3.7. 3β-Acetyl-16α-hydroxyoleanane-13β, 28-olide (4)

Crystalline solids from CH_2Cl_2–MeOH mixture, m.p. 241–242 °C. $[\alpha]_D^{25} = +24°$ ($c = 1.0$, CH_2Cl_2). IR ν_{max} (KBr) cm^{-1}: 3450 (OH), 2925, 2850, 1760, 1730 (ester), 1375, 1100, 1090 (C–O–C), 880. ^{1}H NMR ($CDCl_3$,500 MHz) δ: 4.50 (1H, dd, $J = 11.0$, 5.0 Hz, H-3), 3.90 (1H, d, $J = 5.4$ Hz, H-16), 2.19 (1H, dd, $J = 14.6$, 12.4 Hz, H-19ax), 2.05 (1H, dd, $J = 15.0$, 5.3 Hz, H-15 eq), 2.01 (3H, s, OAc), 1.88 (1H, m, H-22ax), 1.76 (1H, ddd, $J = 13.6$, 13.6, 4.8 Hz, H-15ax), 1.70-1.40 (8H, m, CH_2-2, H-6, CH_2-7, H-8, H-19eq, H-11), 1.46 (1H, m, H-18), 1.30–1.39 (3H, m, H-11, H-12, H-6), 1.25 (1H, ddd, $J = 14.4$, 5.3, 3.6 Hz, H-22eq), 1.18 (3H, s, Me-27), 1.05 (3H, s, Me-25), 0.96 (3H, s, Me-26), 0.92 (3H, s, Me-23), 0.88 (3H, s, Me-30), 0.86 (3H, s, Me-29), 0.81 (3H, s, Me-24), 0.76 (1H, dd, $J = 11.8$, 2.4 Hz, H-5). ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Table 1. CIMS m/z (%): 514 $[M]^+$ (6), 496 $[M-H_2O]^+$ (13), 454 $[M-HOAc]^+$ (15), 436 $[M-H_2O-HOAc]^+$ (100), 264 $[C_{16}H_{24}O_3]^+$ (25), 250 (25), 249 (36), 246 (5), 220 (4), 219 (3), 207 (65), 210 (13), 189 (41). HRMS m/z 514.3658 (calcd for $C_{32}H_{50}O_5$, 514.3580).

3.8. 3β-Acetyl-28-hydroxy-16-oxo-12-oleanene (3β-Acetylschimperinone) (5)

Colourless crystals from petroleum ether–CH_2Cl_2, m.p. 231–233 °C. $[\alpha]_D^{25} = -32°$ (c = 1.0, CH_2Cl_2). IR ν_{max}(KBr) cm^{-1} : 3450 (OH), 2930, 2850, 1732 (ester), 1700 (C=O), 1647 (C=C), 1460, 1380, 1280, 1020, 990, 780. ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 5.30 (1H, t, $J = 3.4$ Hz, H-12), 4.70 (1H, t, $J = 10.5$, 5.1 Hz, H-3), 4.20 (1H, dd, $J = 11.1$ Hz, H-28a), 3.78 (1 H, d, $J = 11.1$ Hz, H-28 b), 2.70 (1H, d, $J = 16.2$ Hz, H-15eq), 2.20 (1 H, ddd, $J = 12.7$, 5.0, 2.6 Hz, H-22eq), 2.15 (1 H, dd, $J = 11.6$, 2.6 Hz, H-18), 1.94 (1 H, ddd, $J = 18.7$, 12.0, 3.4 Hz, H-11eq), 1.85 (1H, ddd, $J = 16.5$ Hz, H-7ax), 1.80 (1H, ddd, $J = 18.7$, 12.4, 3.2 Hz, H-11ax), 1.77 (1H, d, $J = 16.2$ Hz, H-15), 1.75 (1H, dt, $J = 13.6$, 5.0 Hz, H-1ax), 1.68 (1H, t, $J = 14.2$ Hz, H-19ax), 1.65 (1H, m, H-2), 1.60 (1H, m,

H-2), 1.56-1.52 (2H, m, H-6 and H-7), 1.50 (1H, m, H-21), 1.43 (1 H, m, H-60), 1.20 (1H, m, H-21), 1.07 (1H, m, H-19ax), 1.25 (3H, s, Me-27), 1.00 (2H, m, H-9 and H-22), 0.97 (3H, s, Me-25), 0.96 (3H, s, Me-26), 0.89 (3H, s, Me-23), 0.87(3H, s, Me-30), 0.85 (1H, m, H-1), 0.82 (3H, s, Me-29), 0.80 (3H, s, Me-24), 0.77 (1H, dd, $J = 12.0$, 2.3 Hz, H-5). ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Table 1. CIMS *m/z* (%): 498 $[M]^+$ (3), 483 $[M–Me]^+$ (3), 480 $[M–H_2O]^+$ (9), 466 $[M–CH_2OH]^+$ (4), 456 (10), 438 [$M–HOAc]^+$ (6), 423 $[M–HOAc–Me]^+$ (12), 407 $[M–HOAc–CH_2OH]^+$ (7), 249 (100), 248 (70), 235 (11), 231 (20), 219 (25), 218 (5), 217 (4), 207 (17), 202 (20), 189 (20), 169 (10), 168 (12):. HRMS *m/z* 498.2340 (calcd for $C_{32}H_{50}O_4$, 498.2304).

3.9. 3β, 28-Di-O-acetyl-16α-hydroxy-12-oleanene (3β, 28-diacetylprimulagenin A) (6)

Colourless crystals, m.p. 214–216 °C. $[\alpha]_D^{25} = -22°$ ($c = 1.0$, CH_2Cl_2). IR ν_{max} (KBr) cm^{-1}: 3340 (OH), 2920, 2850, 1725 (ester), 1704 (C=O), 1641 (C=C), 1470, 1375, 1210, 1111, 1025, 985, 880, 770. ^{1}H NMR ($CDCl_3$, 500 MHz,) δ: 5.16 (1H, t, $J = 3.6$ Hz, H-12), 4.65 (1H, t, $J = 12.0$, 5.1 Hz, H-3), 4.30 (1H, dd, $J = 11.4$, 5.3 Hz, H-28a), 3.94 (1H, d, $J = 5.5$ Hz, H-16), 3.76 (1H, dd, $J = 11.4$, 3.7 Hz, H-28b), 2.73 (1H, d, $J = 15.8$ Hz, H-15eq), 2.24 (1H, dd, $J = 11.7$, 3.3 Hz, H-18), 2.16 (1H, ddd, $J = 12.1$, 4.7 H-22eq), 2.03 (3H, s, OAc), 2.01 (3H, s, OAc), 1.98 (1H, ddd, $J = 17.8$, 7.0, 3.6 Hz, H-11eq), 1.90 (1H, m, H-7ax), 1.86 (1H, ddd, $J = 18.8$, 11.60, 4.0 Hz, H-11ax), 1.83 (1H, d, $J = 16.0$ Hz, H-15), 1.75–1.10 (8H, m, CH_2-1, CH_2-2, H-19, H-6, H-7, H-21), 1.20 (3H, s, Me-27), 1.05 (3H, s, Me-25), 0.96 (3H, s, Me-26), 0.94 (3H, s, Me-23), 0.92 (3H, s, Me-30), 0.90 (3H, s, Me-29), 0.86 (3H, s, Me-24), 0.75 (1H, dd, $J = 12.6$, 3.5 Hz, H-5): ^{13}C NMR ($CDCl_3$,125 Hz) data, see Table 1. CIMS *m/z* (%): 542 $[M]^+$ (6), 527 $[M\text{-}Me]^+$, 482 $[M–HOAc]^+$ (21), 480 (2), 479 $[M–CH_2OAc]^+$(23), 467 $[M–HOAc–Me]^+$ (11), 409 $[M–HOAc–CH_2OAc]^+$ (9), 381 (5), 292 (45), 249 (18), 217 (12), 216 (13), 202 (3), 201 (2), 189 (70), 183 (1), 133 (30), 43 (100). HRMS *m/z* 542.3971 (calcd for $C_{34}H_{54}O_5$, 542.3893).

3.10. 3β-Acetyl-11α,28-dihydroxy-16-oxo-12-oleanene (3β-acetyl-11α, 28 dihydroxyschimperone) (7)

Colourless crystals from CH_2Cl_2–MeOH (19:1), m.p. > 250 °C; $[\alpha]_D^{25} = -162°$ ($c = 0.5$, MeOH). IR ν_{max} (KBr) cm^{-1}: 3530 (OH), 2925, 2850, 1734 (ester), 1703 (C=O), 1640 (C=C), 1480, 1380, 1210, 1111, 1030, 990, 770. ^{1}H NMR ($CDCl_3$, 500 Hz) δ: 5.15 (1H, t, $J = 4.1$ Hz, H-12), 4.90 (1H, t, $J = 11.6$, 5.4 Hz, H-3), 4.20 (1 H, dd, $J = 11.2$, 5.6 Hz, H-28a), 3.95 (1H, d, $J = 11.2$, 3.6 Hz, H-28b), 3.93 (1H, d, $J = 3.5$ Hz, H-11), 2.73 (1H, d, $J = 16.6$ Hz, H-15eq), 2.27 (1H, dd, $J = 11.6$, 2.8 Hz, H-18), 2.10 (1H, ddd, $J = 13.0$, 4.8, 3.0 Hz, H-22eq), 2.07 (3H, s, OAc), 2.00 (1H, m, H-11eq), 1.88 (1H, m, H-7ax), 1.85 (1H, m, H-11ax), 1.82 (1H, d, $J = 16.6$ Hz, H-15), 1.70 (1 H, t, $J = 14.4$ Hz, H-19ax), 1.68–1.02 (10H, m, CH_2-1, CH_2-2 CH_2-6 and CH_2-7, H-19, H-9), 1.21 (3H, s, Me-27), 1.05 (3H, s, Me-25), 1.00 (3H, s, Me-26), 0.97 (3H, s, Me-23), 0.94 (3H, s, Me-30), 0.92 (3H, s, Me-29), 0.84 (3H, s, Me-24), 0.85 (1H, m, H-1), 0.63 (1H, m, H-5). ^{13}C NMR ($CDCl_3$,125 Hz) data, see Table 1. CIMS *m/z* (%). 514 $[M]^+$ (5), 499 $[M–Me]^+$ (3), 496 $[M–H_2O]^+$ (4), 481 $[M–H_2O\text{-}Me]^+$ (23), 454 $[M–HOAc]^+$ (10), 439 $[M–HOAc–Me]^+$ (7), 436 $[M–H_2O–HOAc]^+$ (11), 421 $[M–H_2O–HOAc–Me]^+$ (100), 357, 265 $[C_{16}H_{25}O_3]^+$ (22), 249 (10), 248 (6), 234 (2), 217 (6), 216 (8), 207 (100), 203 (40), 202 (13), 189 (19), 46 (65). HRMS *m/z* 514.3658 (calcd for $C_{32}H_{50}O_5$, 514.3580).

3.11. 3β, 11α, 16α, 28-Tetrahydroxy-12-oleanene (11α-hydroxyprimulagenin A) (8)

Colourless crystals, m.p. >250 °C. $[\alpha]_D^{25} = +70°$ ($c = 0.6$, MeOH). IR ν_{max} (KBr) cm^{-1}: 3500–3000 (OH), 2920, 2850, 1732 (ester), 1700 (C=O), 1647 (C=C), 1460, 1380, 1280, 1020, 990, 780. ^{1}H NMR ($CDCl_3$+ drop DMSO-d_6, 500MHz) δ: 5.24 (1H, t, $J = 2.8$ Hz, H-12), 4.30 (1H, dd, $J = 9.2$, 6.6 Hz, H-28a), 4.00 (1H, dd, $J = 8.7$, 3.4 Hz, H-11), 3.97 (1H, dd, $J = 9.2$, 4.0 Hz, H-28b), 3.85 (1H, d, $J = 5.7$ Hz, H-16), 3.65 (1H, t, $J = 11.5$, 5.3 Hz, H-3), 2.35 (1H, dd, $J = 15.1$, 5.4 Hz, H-15eq), 2.16 (1H, dd, $J = 12.3$, 3.2 Hz, H-18), 2.08 (1H, ddd, $J = 13.6$, 5.0, 2.5 Hz, H-22eq), 1.90 (1H, ddd, $J = 18.7$, 12.0, 3.4 Hz, H-7ax), 1.84 (1H, d, $J = 16.1$ Hz, H-15ax), 1.77 (1H, d, $J = 16.2$ Hz, H-15), 1.76 (1H, dt, $J = 14.4$, 4.8 Hz, H-1ax), 1.72 (1H, t, $J = 14.8$ Hz, H-19ax), 1.70-1.30 (8H, m, CH_2-2, CH_2-6, H-7, CH_2-21, H-9), 1.26 (3H, s, Me-27), 1.10 (2H, m, H-9 and H-22), 1.07 (1H, m, H-19eq), 1.03 (3H, s, Me-25), 1.00 (3H, s, Me-23), 0.97 (3H, s, Me-26), 0.94 (3H, s, Me-30), 0.88 (1H, m, H-1), 0.90 (3H, s, Me-29), 0.82 (3H, s, Me-24), 0.65 (1H, dd, $J = 12.0$, 2.2 Hz, H-5). ^{13}C NMR (drop DMSO-d_6+$CDCl_3$,125 MHz) data, see Table 1. CIMS *m/z* (%): 474 $[M]^+$ (2), 456 $[M–H_2O]^+$(5), 459 $[M–Me]^+$ (7), 266 $[C_{16}H_{26}O_3]^+$ (13), 248 (17), 235 $[C_{15}H_{23}O_2]^+$ (10), 218 (25), 217 $[C_{15}H_{21}O]^+$ (15), 208 $[C_{14}H_{24O}]^+$ (15), 207 $[C_{14}H_{24}O]^+$ (20), 203 (30), 189 $[C_{14}H_{21}]^+$ (100): HRMS *m/z* 474.3709 (calcd for $C_{30}H_{50}O_4$, 474.3632).

3.12. 3β-Acetyl-16α, 28α-dihydroxy-13β, 28-oxydooleanane (9)

Colourless crystals from CH_2Cl_2-MeOH, m.p. 243–245 °C. $[\alpha]_D^{25} = -94°$ ($c = 0.6$, CH_2Cl_2); IR ν_{max} (KBr) cm^{-1}: 3452 (OH), 1740, 1734 (ester), 1385, 1375, 1090 (C–O–C). ^{1}H NMR ($CDCl_3$+ drop DMSO-d_6, 500 MHz) δ: 4.80 (1H, br s, H-28), 4.70 (1H, dd, $J = 10.5$, 5.1 Hz, H-3), 4.12 (1H, d, $J = 3.5$ Hz, H-16), 2.53 (m, H-19eq), 2.05 (3H, s, OAc), 2.01 (1H, m, H-19ax), 1.86 (1H, dd, $J = 13.80$, 5.8 Hz, H-15ax), 1.76 (1H, m, H-2ax), 1.72 (1H, m, H-1eq), 1.69 (1H, m, H-18), 1.54 (1H, m, H-

2eq), 1.48-1.34 (7H, m, CH_2-6, H-7, H-9, H-11, H-12eq, H-19eq), 1.30–1.00 (6H, m, H-1, H-7, H-9, H-11, H-12, H-15 eq), 1.25 (3H, s, Me-27), 1.03 (3H, s, Me-25), 0.98 (3H, s, Me-23), 0.96 (3H, s, Me-26), 0.91 (3H, s, Me-30), 0.88 (3H, s, Me-29), 0.84 (3H, s, Me-24), 0.80 (1H, m, H-5). ^{13}C NMR ($CDCl_3$+ DMSO-d_6, 125MHz,) data, see Table 1. CIMS *m/z* (%): 514 $[M]^+$ (7), 496 $[M-H_2O]^+$ (8), 481 $[M-Me]^+$ (3), 474 (12), 454 $[M-HOAc]^+$(100), 438 (10), 395 (21), 278 (30), 264 (28), 249 (30), 233 (14), 224 (1), 215 (15), 207 (70), 189 (10), 81 (42), 69 (54), 55 (59). HRMS *m/z* 516.3815 (calcd for $C_{32}H_{52}O_5$, 516.3736).

3.13. 3β, 28α-Dihydroxy-16-oxo-13β, 28-oxydooleanane (*10*)

Colourless crystals from CH_2Cl_2–MeOH (9:1), m.p. >250 °C. $[\alpha]_D^{25} = -65°$ ($c = 1.0$, CH_2Cl_2). IR ν_{max} (KBr) cm^{-1}: 3450 (OH), 1760 (C=O), 1485, 1375, 1100 (C–O–C). ^{1}H NMR ($CDCl_3$,+ drop DMSO-D_6, 500 MHz) δ: 5.28 (1H, s, H-28), 3.60 (1H, dd, $J = 10.6$, 5.2 Hz, H-3), 2.20 (1H, dd, $J = 12.9$, 4.0 Hz, H-18), 2.26 (1H, dd, $J = 13.80$, 5.8 Hz, H-15ax), 2.09 (1H, ddd, $J = 13.6$, 13.6, 5.3 Hz, H-19ax), 1.94 (1H, m, H-2ax), 1.91 (1H, m, H-11ax), 1.88 (1H, m, H-2eq), 1.70 (1H, m, H-1eq), 1.68 (1H, m, H-7ax), 1.64 (1H, m, H-12eq), 1.62 (1H, m, H-15 eq), 1.60-1.35 (4H, m, CH_2-6, H-11eq, H-19eq), 1.0 (1H, m, H-1ax), 1.25 (3H, s, Me-27), 1.03 (3H, s, Me-25), 0.98 (3H, s, Me-23), 0.96 (3H, s, Me-26), 0.91 (3H, s, Me-30), 0.86 (3H, s, Me-29), 0.84 (3H, s, Me-24), 0.76 (1H, m, H-5). ^{13}C NMR ($CDCl_3$,+ drop DMSO-d_6, 500 MHz) data, see Table 1. CIMS *m/z* (%): 472 $[M]^+$ (5), 454 $[M-H_2O]^+$ (20), 276 (56), 265 (22), 264 (11), 231 (23), 224 (13), 207 $[C_{14}H_{23}O]^+$ (100), 189 (55), 85 (46), 69 (51), 42 (88). HRMS *m/z* 472.3553 (calcd for $C_{30}H_{48}O_4$, 472.3474).

3.14. 3β, 16α-dihydroxy-13β, 28-epoxyoleanane (protoprimulagenin A) (*11*), 3β-Hydroxy-16-oxo-13β,28-epoxyoleanane (aegicerin) (*12*), 3β,16-dioxo-13β,28-epoxyoleanane (embelinone) (*13*), and 3β,28-dihydroxy-16-oxo-12-oleanene (schimperinone) (*14*)

Compounds **11**–**14** had spectroscopic data (IR, ^{1}H and ^{13}C NMR, and MS) similar to those previously reported (Bittner et al., 2003; Venkateswara, 1964; Baigent et al., 1976; Doajing et al., 2005).

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