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Pentacyclic triterpenoids from *Embelia schimperi*

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Abstract

Five oleanane-type pentacyclic triterpenoids were isolated by chromatographic separation of a chloroform extract of the stem bark of *Embelia schimperi*. Three of these compounds have a methyleneoxy bridge. Two compounds, embelinone and schimperinone, are reported here for the first time from a natural source (they have been synthesized previously during chemical transformations). Their structures were determined by spectroscopic techniques, among which 2-D NMR was useful for complete characterization. Three of the triterpenoids exhibited mild antibacterial properties against the gram-positive bacterial strain *Rhodococcus* sp.

Keywords: *Embelia schimperi*; Myrsinaceae; Pentacyclic triterpenoids; Embelinone; Schimperinone; Aegicerin; Protoprimulagenin A; Primulagenin A; Antibacterial activity

1. Introduction

Embelia schimperi Vatke is one of the five known species of the family Myrsinaceae found in Kenya (Beerntje, 1994). The fruits of the plant are used by the Maasai as an antibacterial and anthelminthic remedy, especially against tapeworm, *Taenia saginata* (Kokwaro, 1976), and these biological activities have been supported by systematic studies (Boegh et al., 1996). Recent studies have shown that a methanolic extract of the fruit has inhibitory effects on hepatitis C protease (Hussein et al., 2000). Previous phytochemical studies reported the isolation of long alkyl chain substituted benzoquinones (Midiwo et al., 1988). Among them is embelin, a compound with a wide spectrum of biological and pharmacological properties. Anthraquinones and flavonoids have been also isolated from the berries and the leaves, respectively (Midiwo and Arot, 1993; Arot and Williams, 1997).

In the present study, we report the isolation of five pentacyclic triterpenoids (PCTTs) of oleanane type from the chloroform extract of the stem bark of *E. schimperi*. Three of them contain a methyleneoxy (CH_2O) bridge, while the other two show unsaturation. Embelinone (**1**) and schimperinone (**4**) are reported here for the first time from a natural source. Aegicerin (**2**) was initially reported from *Aegiceras majus* Gaertn (Myrsinaceae), but NMR spectral data were lacking in the study (Rao, 1964). In the current study, spectral data for protoprimulagenin A (**3**) were improved, and the structure was verified. It is interesting to note that saponins of **3** and primulagenin A (**5**) with four or five sugar moieties linked at position 3 exhibited strong molluscicidal and antifungal properties (Ohtani et al., 1993; Kohda et al., 1989).

2. Results and discussion

Isolation and purification of the compounds was accomplished by repeated column and preparative thin layer chromatographic techniques. ^{13}C NMR spectral data revealed that the five compounds each contained 30 carbon atoms, which is characteristic of triterpenoids (Table 1). All seven methyl signals appeared as singlets in the ^{1}H NMR spectra, indicating that they were isolated and that the spectral patterns were consistent with PCTT skeletal structure (Mahato and Kundu, 1994).

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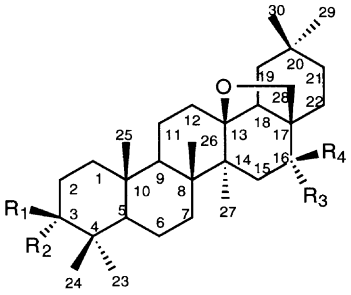
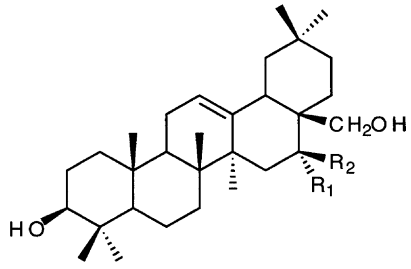


1. $R_1 + R_2 = O$; $R_3 + R_4 = O$
2. $R_1 = OH$; $R_2 = H$; $R_3 + R_4 = O$
3. R_1, $R_3 = OH$; R_2, $R_4 = H$



4. $R_1 + R_2 = O$
5. $R_1 = OH$; $R_2 = H$

Table 1
^{13}C NMR data for **1**–**5** (125 MHz, $CDCl_3$)

C No.	1	2	3	4	5
1	39.7 *t*	39.9 *t*	38.7 *t*	39.1 *t*	38.9 *t*
2	34.1 *t*	27.3 *t*	27.3 *t*	27.8 *t*	28.0 *t*
3	217.7 *s*	78.9 *d*	79.0 *d*	79.0 *d*	78.9 *d*
4	47.4 *s*	38.9 *s*	38.9 *s*	38.8 *s*	38.9 *s*
5	54.8 *d*	54.3 *d*	55.1 *d*	55.1 *d*	55.3 *d*
6	18.9 *t*	17.6 *t*	17.7 *t*	17.8 *t*	18.1 *t*
7	31.6 *t*	33.5 *t*	31.0 *t*	31.9 *t*	32.2 *t*
8	42.5 *s*	42.6 *s*	42.0 *s*	41.0 *s*	40.7 *s*
9	49.2 *d*	49.9 *d*	50.1 *d*	48.3 *d*	48.8 *d*
10	36.6 *s*	36.9 *s*	36.9 *s*	36.8 *s*	36.9 *s*
11	18.9 *t*	18.5 *t*	18.6 *t*	24.3 *t*	24.1 *t*
12	32.9 *t*	31.6 *t*	34.0 *t*	122.4 *d*	122.6 *d*
13	86.1 *s*	86.2 *s*	86.4 *s*	145.6 *s*	145.3 *s*
14	49.7 *s*	49.6 *s*	43.9 *s*	47.8 *s*	42.5 *s*
15	45.3 *t*	45.4 *t*	36.8 *t*	45.3 *t*	35.7 *t*
16	213.3 *s*	213.7 *s*	77.4 *d*	213.2 *s*	73.2 *d*
17	56.0 *s*	56.0 *s*	44.1 *s*	55.7 *s*	41.2 *s*
18	54.4 *d*	55.0 *d*	50.6 *d*	48.6 *d*	48.3 *d*
19	39.9 *t*	38.9 *t*	38.8 *t*	46.3 *t*	46.5 *t*
20	31.7 *s*	31.6 *s*	31.5 *s*	31.8 *s*	31.6 *s*
21	35.1 *t*	35.2 *t*	36.6 *t*	35.5 *t*	37.1 *t*
22	34.7 *t*	24.7 *t*	32.3 *t*	28.7 *t*	31.3 *t*
23	26.4 *q*	27.9 *q*	27.9 *q*	27.8 *q*	28.2 *q*
24	21.0 *q*	15.9 *q*	16.1 *q*	15.9 *q*	16.0 *q*
25	15.6 *q*	15.3 *q*	15.3 *q*	15.8 *q*	15.5 *q*
26	18.1 *q*	18.5 *q*	18.1 *q*	18.6 *q*	18.0 *q*
27	21.4 *q*	21.6 *q*	19.4 *q*	28.6 *q*	27.1 *q*
28	75.1 *t*	75.1 *t*	78.1 *t*	71.2 *t*	70.5 *t*
29	33.2 *q*	33.3 *q*	33.4 *q*	33.4 *q*	33.6 *q*
30	23.5 *q*	23.5 *q*	24.3 *q*	23.7 *q*	24.8 *q*

C-multiplicities were determined by DEPT data.

Compounds **1**, **2** and **3** each have 11 methylene carbons, while **4** and **5** each have 10. COSY and HMQC spectra assisted in the assignment and pairing up of these protons.

Compounds **1** and **2** were obtained in the form of a white powder and crystallised from $CHCl_3$/MeOH (1:4) to yield colourless crystals. HRMS showed that they had molecular masses of 454 and 456, respectively. IR spectra exhibited carbonyl absorption at around 1703 cm^{-1}; this absorption was more intense in **1** suggesting more than one carbonyl group. The NMR spectral pattern for compounds **1** and **2** was similar to that for compound **3**, and assignment was based on this compound (Kohda et al., 1989). The compounds showed an oxygenated quaternary carbon at δ 86, which represented the fingerprint for the PCTTs with a methyleneoxy bridge between C-13 and C-17 (Mahato and Kundu, 1994). The chemical shifts due to the methylene of the bridge appeared at δ 76.7 and 76.5 for **1** and **2**, respectively. The compounds exhibited IR absorption associated with the C–O–C group at 1121, 1080 and 1045 cm^{-1} (Rao, 1964).

The ^{13}C NMR spectrum of **1** showed two downfield shifts at δ 213.3 and 217.7, while **2** exhibited only one downfield shift at δ 213.7, which was assigned to the carbonyl at position 16. This assignment, based on **3**, was due to the relative effect exerted by the carbonyl group in **2** and the hydroxyl group in **3** on the two bridge protons. The signal in the ^1H NMR spectrum in **3** attributed to the *endo* proton appeared at δ 3.10, but the signal was shifted downfield to δ 3.82 in **2**. This implied that there was an oxygenated centre near the bridge in both compounds, but with a different influence on the methyleneoxy hydrogens (Kohda et al., 1989). The methylene protons at position 15 in **2** appeared as two clear doublets, and one of them was noticeably shifted downfield. The ^{13}C NMR spectrum of **2** showed an oxygenated methine centre, assignable to position 3. This was lacking in the spectrum of **1**, which indicated that the latter was an oxidized derivative of **2**. This accounted for the more deshielded carbonyl signal (Mahato and Kundu, 1994). The placement was supported by the deshielding effect on both C-2 and its protons. A change in the splitting pattern of these protons was also noted (Alam et al., 2000). The hydroxyl group at position 3 of **2** was assigned equatorial (β) orientation due to the fact that the geminal proton centred at δ 3.13 appeared as a *dd* ($J = 11.5$ and 5.0 Hz)

and was thus assigned axial (α) orientation (Quijano et al., 1998).

Compound **4**, which was isolated as colourless crystals, had a molecular mass of 456. NMR spectral data were closely related to those of the earlier reported **5** (Ohtani et al., 1993), However, the ^{13}C NMR spectrum of **4** showed a downfield shift at δ 213.6 and lacked one of the oxygenated methine carbons present in the spectrum of **5**. This suggested that the former was an oxidised derivative of **5** at position 16. The presence of a double bond was evident in the spectra. It involved a quaternary carbon atom, since only one signal was observed in the ^1H NMR at δ 5.29, appearing as a triplet ($J = 3.5$ Hz), and was thus assigned between C-12 and C-l3, like in **5** (Ohtani et al., 1993; Quijano et al., 1998). The oxygenated methylene carbon appeared in the ^{13}C NMR spectrum upfield at δ 70.5, and the signals associated in the ^1H NMR spectrum had a larger coupling constant (10.5 Hz) than that of the bridge methylene protons of **1**, **2** and **3**. This suggested a terminal CH_2OH group (Mahato and Kundu, 1994; Ohtani et al., 1993). All the other assignments were based on **5** and corroborated by 2-D NMR experiments.

The bridged triterpenoids exhibited mild antibacterial activity against the gram-positive strain of *Rhodococcus* sp. Compounds **1**, **2** and **3** showed an average growth inhibition (lytic zones) of 1.0, 1.1 and 1.8 cm at a concentration of 50 μg/ml. The antibiotic, ampicillin, used as the standard, had an average growth inhibition of 3.0 cm. No activity was noted for the other two triterpenoids. No antibacterial activity was observed towards cultures of *Escherichia coli, Pseudomonas purida* and *Bacillus subtili*.

3. Experimental

3.1. General experimental procedures

Melting points were determined using Thomas Hoover capillary apparatus and are uncorrected. IR spectra were recorded on a Nicolet 5ZDX FT-IR Spectrometer. ^1H, ^{13}C and 2-D NMR spectra were obtained on a Brüker WP 500 Spectrometer with $CDCl_3$ as the solvent and TMS as internal standard. MS were obtained using a Finnigan 4020 quadropole high-resolution mass pectrometer. Silica gel 60 (0.063–0.200 mm) was used for column chromatography. Analytical and preparative TLC of 0.25 mm thickness were carried out using Merck silica gel 60 F_{254} on aluminium and glass, respectively. Each preparative TLC plate was loaded with a maximum 40 mg of sample. The spots representing the triterpenoids were followed by spraying the chromatograms with 5% H_2SO_4 in MeOH then heating to 110 °C.

3.2. Plant material

Stem bark of *E. schimperi* was collected from Ngong Hills about 30 km south of Nairobi in March 2001 by one of the authors (P.C.K.), and authentication was performed at the Moi University Herbarium, Eldoret, Kenya. A voucher specimen (PCK/004/2001) has been deposited in the same herbarium.

3.3. Isolation and extraction

The stem bark of *E. schimperi* (2.5 kg) was ground and macerated with MeOH (2 1) at room temperature for 48 h, and the process was repeated twice. The extracts were combined and concentrated under reduced pressure. The resulting brown gummy residue (25 g) was extracted twice with $CHCl_3$ (200 ml). The extracts were combined and concentrated under reduced pressure to give a dark green residue (4 g). This $CHCl_3$ extract was subjected to chromatographic separation on silica gel (100 g) using a gradient of *n*-hexane–EtOAc (100:0–0:100) and then a gradient of EtOAc–MeOH (100:0–90:10) as the eluting solvent systems. Four major fractions were obtained. Fraction 2 (1.4 g) showed presence of the triterpenoids and was further chromatographed on silica gel (40 g) using a gradient of *n*-hexane–EtOAc (100:0–0:100), from which three subfractions of interest were obtained. Compounds from subfraction 2-1 (270 mg) were first purified by column chromatography on silica gel (15 g) using CH_2Cl_2 as the eluent. This was followed by prep. TLC, in which development of the plates was carried out with CH_2Cl_2. Compounds **1** (21 mg) and **4** (13 mg) were isolated from this fraction. Subfraction 2-2 (190 mg) was subjected to prep. TLC eluted with 1% MeOH in CH_2Cl_2 to yield **2** (24 mg), **3** (31 mg) and **5** (15 mg). Subfraction 2-3 (197 mg) showed two strong spots and the compounds precipitated in MeOH. The ppt. was dissolved in CH_2Cl_2 and the two compounds were separated by prep. TLC eluted with 1% MeOH in CH_2Cl_2 to yield **2** (24 mg) and **3** (29 mg). Colourless crystals of the triterpenoids were obtained by recrystallization from $CHCl_3$–MeOH (1:4).

3.4. Embelinone (*1*), (3,16-dioxo-13β: 17-methylene-oxyoleanane)

Clear needles, mp 257–259 °C, $[\alpha]_D^{25}$ −4° ($CHCl_3$, *c* 0.6). IR ν_{max} (film on $CHCl_3$) cm^{-1}: 2949, 2859, 1704 (C=O), 1436, 1384, 1233, 1121 (C–O–C), 1080 (C–O–C), 1045 (C–O–C), 1045, 990. ^1H NMR (500 MHz, $CDCl_3$): δ 0.79 (3H, *s*, Me-30), 0.87 (3H, *s*, Me-29), 0.93 (3H, *s*, Me-25), 0.97 (6H, *s*, Me-23 and Me-27), 1.01 (3H, *s*, Me-24), 1.10–1.14 (2H, *m*, H-l2 and H-22), 1.15–1.19 (2H, *m*, H-9 and H-21), 1.21 (3H, *s*, Me-26), 1.24 (1H, *m*, H-5), 1.26–1.31 (3H, *m*, H-1 and 2H-19), 1.35–1.39 (2H, *m*, H-6 and H-12), 1.46–1.50 (2H, *m*, H-6 and

H-7), 1.51–1.53 (2H, *m*, H-11eq and H-21), 1.71 (1H, *ddd, J* = 4.9, 13.5, 17.9 Hz,H-11ax), 1.81 (1H, *d, J* = 16.0 Hz, H-15), 1.87 (1H, *dd, J* = 2.5, 11.3 Hz, H-18), 1.90 (1H, *m*, H-7), 1.94 (1H, *m*, H-1), 2.09 (1H, *ddd, J* = 2.5, 5.1, 13.3 Hz, H-22eq), 2.34 (lH, *ddd, J* = 4.2, 7.2, 15.7 Hz, H-2eq), 2.47 (1H, *ddd, J* = 7.4, 10.3, 15.7 Hz, H-2ax), 2.65 (1H, *d, J* = 16.0 Hz, H-15), 3.40 (1H, *d, J* = 8.3 Hz, H-28), 3.82 (1H, *d, J* = 8.3 Hz, H-28). ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1. EIMS 70 eV, *m*/*z* (rel. int.): 454 $[M]^+$ (100), 424 $[M–CH_2O]^+$ (19), 383 (6), 269 (7), 248 (42), 235 (37) 219 (12), 203 (34). HR-MS *m*/*z*: 454.343384 (calc. for $C_{30}H_{46}O_3$, 454.344696).

3.5. Aegicerin (**2**), (3β-hydroxy-13β:17-methyleneoxy-16-oxooleanane)

Clear needles, mp 252–255 °C, $[\alpha]_D^{25}$ −24.5° ($CHCl_3$, *c* 0.86). IR ν_{max} (film on $CHCl_3$) cm^{-1}: 3382 (OH), 2951, 2864, 1701 (C=O), 1456, 1386, 1216, 1122 (C–O–C), 1077 (C–O–C), 1033 (C–O–C), 990, 757. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.60 (1H, *dd, J* = 11.5, 2.0 Hz, H-5), 0.71 (3H, *s*, Me-25), 0.79 (3H, *s*, Me-30), 0.82 (3H, *s*, Me-24), 0.85 (1H, *m*, H-1), 0.87 (3H, *s*, Me-29), 0.91 (3H, *s*, Me-23), 0.96 (3H, *s*, Me-27), 1.05 (1H, *m*, H-12), 1.10 (2H, *m*, H-9 and H-22), 1.16 (3H, *s*, Me-26), 1.16 (1H, *m*, H-21), 1.29 (1H, *t, J* = 13.7 Hz, H-19ax), 1.31 (1H, *m*, H-19eq), 1.36–1.39 (2H, *m*, H-6 and H-12), 1.41–1.45 (2H, *m*, H-6 and H-7eq), 1.46–1.48 (2H, *m*, H-11eq and H-21), 1.55 (2H *m*, 2H-2), 1.62 (1H, *ddd, J* = 3.6, 11.0, 18.1 Hz, H-11ax), 1.68 (1H, *dt, J* = 4.5, 13.2 Hz H-1ax), 1.79 (1H, *d, J* = 16.1 Hz, H-15), 1.88 (1H, *ddd, J* = 3.5, 12.5, 17.3 Hz, H-7ax), 1.89 (1H, *dd, J* = 3.0, 11.5 Hz, H-18), 2.07 (1H, *ddd, J* = 2.4, 5.0, 13.5 Hz, H-22eq), 2.63 (1H, *d, J* = 16.1 Hz, H-15), 3.13 (1H, *dd, J* = 5.0, 11.4 Hz, H-3), 3.39 (1H, *d, J* = 8.3 Hz, H-28), 3.82 (1H, *d, J* = 8.3 Hz, H-28). ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1. EIMS 70 eV, *m*/*z* (rel. int.): 456 $[M]^+$ (43), 439 $[M-OH]^+$ (24), 426 $[M-CH_2O]^+$ (15), 248 (71), 235 (100) 217 (34), 202 (58). HR-MS *m*/*z*: 456.358231 (calc. for $C_{30}H_{48}O_3$, 454.360346).

3.6. Protoprimulagenin (**3**), (3β,16α-dihydroxy-13β:17 methyleneoxyoleanane)

Clear needles, mp 269–271 °C, $[\alpha]_D^{25}$ +16° ($CHCl_3$, *c* 0.75). IR ν_{max} (film on $CHCl_3$) cm^{-1}: 3411 (OH), 2923, 2859, 1446, 1385, 1363, 1302, 1258, 1183, 1117 (C–O–C), 1097 (C–O–C), 1036 (C–O–C), 980, 949, 882. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.61 (1H, *dd, J* = 11.5, 2.0 Hz, H-5), 0.70 (3H, *s*, Me-25), 0.80 (3H, *s*, Me-30), 0.84 (3H, *s*, Me-24), 0.87 (1H, *m*, H-1), 0.90 (3H, *s*, Me-29), 0.91 (3H, *s*, Me-23), 1.05 (1H, *m*, H-21), 1.09 (3H, *s*, Me-27), 1.12–1.14 (2H, *m*, H-9 and H-21), 1.14 (3H, *s*, Me-26), 1.15 (1H, *m*, H-12), 1.18 (1H, *m*, H-1), 1.26 (1H, *ddd, J* = 3.0, 5.0, 13.7 Hz, H-22eq), 1.33–1.35 (2H, *m*, H-11, H-12), 1.36–1.39 (2H, *m*, H-6 and H-18), 1.41–1.45 (2H, *m*, H-6 and H-7), 1.48–1.53 (3H, *m*, 2H-2, H-l1), 1.67–1.72 (2H, *m*, H-7 and H-19eq), 1.83 (1H, *ddd, J* = 5.0, 13.5, 13.5 Hz, H-15ax), 1.92 (1H, *ddd, J* = 5.3, 13.5, 13.7 Hz, H-22ax), 2.10 (1H, *brdd, J* = 5.2, 14.5 Hz, H-15eq), 2.20 (1H, *dd*, *J* = 12.2, 14.5 Hz, H-19ax), 3.07 (1H, *d, J* = 7.5 Hz, H-28), 3.13 (1H, *dd, J* = 5.0, 11.4 Hz, H-3), 3.43 (1H, *d*, *J* = 7.5 Hz, H-28), 3.91 (1H, *d, J* = 5.4 Hz, H-16). ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1. EIMS 70 eV, *m*/*z* (rel. int.): 458 $[M]^+$ (37), 441 $[M-OH]^+$ (24), 426 $[M-CH_2O-H]^+$ (7), 248 (71), 410 (9), 409 (11), 385 (11), 249 (22), 236 (30), 220 (57), 219 (65), 207 (100), 189 (34). HR-MS *m*/*z*: 458.379227 (calc. for $C_{30}H_{50}O_3$, 458.375996).

3.7. Schimperinone (**4**), (3β,28-dihydroxy-16-oxo-12-oleanene)

Colourless needles, mp 269–271 °C, $[\alpha]_D^{25}$ −11° ($CHCl_3$, *c* 0.60). IR ν_{max} (film on $CHCl_3$) cm^{-1}: 3395 (OH), 2924, 2854, 1706 (C=O), 1642 (C=C), 1591, 1461, 1383, 1281, 1080, 1022, 799. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.62 (1H, *dd, J* = 11.5, 2.0 Hz, H-5), 0.79 (3H, *s*, Me-24), 0.86 (1H, *m*, H-1), 0.87 (3H, *s*, Me-29), 0.90 (3H, *s*, Me-30), 0.94 (3H, *s*, Me-25), 0.96 (3H, *s*, Me-26), 1.02 (3H, *s*, Me-23), 1.09 (1H, *m*, H-19eq), l.22 (3H, *s*, Me-27), 1.10 (2H, *m*, H-9 and H-22), 1.16 (1H, *m*, H-21), 1.39 (H, *m*, H-6), 1.48 (1H, *m*, H-21), 1.51–1.55 (2H, *m*, H-6 and H-7), 1.56 (1H, *m*, H-2), 1.61 (1H *m*, H-2), 1.65 (1H, *t, J* = 13.8 Hz, H-19ax), 1.72 (1H, *dt, J* = *4.7*, 13.5 Hz, H-1ax), 1.81 (1H, *d, J* = 16.1 Hz, H-15), 1.84 (1H, *ddd, J* = 3.5, 12.0, 18.3 Hz, H-11ax), 1.85 (1H, *ddd*, *J* = 4.0, 11.5, 16.8 Hz, H-7ax), 1.95 (1H, *ddd, J* = 3.5, 6.8, 18.4 Hz, H-11eq), 2.10 (1H, *ddd, J* = 2.5, 5.2, 12.6 Hz, H-22eq), 2.14 (1H, *dd, J* = 3.0, 11.5 Hz, H-18), 2.65 (1H, *d*, *J* = 16.1 Hz, H-15), 3.25 (1H, *dd, J* = 5.0, 11.5 Hz, H-3), 3.61 (1H, *d, J* = 10.5 Hz, H-28), 3.83 (1H, *d, J* = 10.5 Hz, H-28), 5.35 (1H, *t, J* = 3.5 Hz, H-12). ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1. EIMS 70 eV, *m*/*z* (rel. int.): 456 $[M]^+$(20), 439 $[M-OH]^+$ (7), 248 (14), 235 (100), 217 (18), 203 (27), 168 (19). HR-MS *m*/*z*: 456.359029 (calc. for $C_{30}H_{48}O_3$, 454.360346).

3.8. Primulagenin A (**5**), (3β,16α,28-trihydroxy-12-oleanene)

Colourless needles, mp 241–244 °C, data in agreement with those reported (Ohtani et al., 1993; Katagawa et al., 1972). ^{13}C NMR (125 MHz, $CDCl_3$): see Table 1.

3.9. Biological activity tests

The potential antibiotic properties of the isolated triterpenoids were investigated by the modified Luria broth (LB) agar plate method of Bhatnagar et al. (1961). For the compound being tested, 5 mg were dissolved in a 0.5

ml of DMSO. A sample of 50 μl of a bacterial culture of *E. coli, P. putida, B. subtilis* or *Rhodococcus* sp., grown in LB liquid medium to the steady state phase (more than 12 h of growth), was spread on the surface of the LB agar plates. The plates were dried for 30 min at room temperature. When no more liquid was observed on the plates, 5 μl of an already prepared solution of the particular test compound was introduced into the agar. The solvent, DMSO, and the well-known antibiotic, ampicillin (at a concentration of 50 μg/ml) were used as controls. The plates were incubated at 30 °C for 24 h, and the growth inhibition was based on the assessment of the lytic zones on the plates.

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References

Alam, M.S., Chopra, N., Ali, M., Niwa, M., 2000. Normethyl pentacyclic and lanostane-type triterpenes from *Adiantum venustum*. Phytochemistry 54, 215–220.

Arot, L.O.M., Williams, L.A.D., 1997. A flavonol glycoside from *Embelia schimperi*. Phytochemistry 44, 1397–1398.

Beerntje, H.J., 1994. Kenya Trees, Shrubs and Lianas. National Museum of Kenya, Nairobi.

Bhatnagar, S.S., Santapau, H., Desa, J.D.H., Maniar, A.C., Ghadially, N.C., Solomon, M.J., Yellore, S., Rao, T.N.S., 1961. Biological activity of Indian medicinal plants: antibacterial, antitubercular and antifungal action. Ind. J. Med. Res. 49, 799–813.

Boegh, H.O., Andreassen, J., Lemmich, J., 1996. Anthelmintic usage of extract of *Embelia schimperi* from Tanzania. J. Ethnopharmacol. 50, 35–42.

Hussein, G., Miyashiro, H., Nakamura, N., Hattori, M., Kakiuchi, N., Shimotohno, K., 2000. Inhibitory effects of Sudanese medicinal plant extracts on hepatitis C virus (HCV) protease. Phytother. Res. 14, 510–516.

Katagawa, I., Matsuda, A., Yosioka, I., 1972. Saponin and sapogenol. VII. Sapogenol constituents of five *Primulaceous* plants. Chem. Pharm. Bull. 20, 2226–2234.

Kohda, H., Takeda, O., Tanaka, S., 1989. Molluscicidal triterpenoidal saponin from *Lysimachia sikokiana*. Chem. Pharm. Bull. 37, 3304–3305.

Kokwaro, J.O., 1976. Medicinal Plants of East Africa. East Africa Literature Bureau, Nairobi.

Mahato, S.B., Kundu, A.P., 1994. ^{13}C NMR spectra of pentacyclic triterpenoids—a complilation and some salient features. Phytochemistry 37, 1517–1575.

Midiwo, J.O., Arot, L.M., 1993. Polynuclear acetogenic pigments in the fruits of the Myrsinaceae. Int. J. Bio. Chem. Phys. 2, 115–116.

Midiwo, J.O., Arot, L.M., Mbakaya, C.L., 1988. Distribution of benzoquinone pigments in Kenyan Myrsinaceae. Bull. Chem. Soc. Ethiop. 2, 83–85.

Ohtani, K., Mavi, S., Hostettmann, K., 1993. Molluscicidal and antifungal triterpenoid saponins from *Rapanea melanophloeos* leaves. Phytochemistry 33, 83–86.

Quijano, L., Rios, T., Fronczek, F.R., Fischer, N.H., 1998. The molecular structure of maniladiol from *Baccharis saiicina*. Phytochemistry 49, 2065–2068.

Rao, K.V., 1964. Chemistry of *Aegiceras majus* Gaertn V—structure of the triterpene aegicerin. Tetrahedron 20, 973–977.