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ACYCLIC TRITERPENOIDS FROM *EKEBERGIA CAPENSIS*

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Key Word Index—*Ekebergia capensis*; Meliaceae; acyclic triterpenoid; 2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosatetraene; 2-hydroxymethyl-2,3,22,23-tetrahydroxy-6,10,15,19,23-pentamethyl-6,10,14,18-tetracosatetraene.

Abstract—From the dried bark of *Ekebergia capensis*, two novel acyclic triterpenoids, 2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosatetraene and 2-hydroxymethyl-2,3,22,23-tetrahydroxy-6,10,15,19,23-pentamethyl-6,10,14,18-tetracosatetraene were isolated, along with known cyclic triterpenoids. The structures of these two new triterpenoids were determined by spectroscopic and chemical methods.

INTRODUCTION

Ekebergia capensis (Meliaceae) is a tall tree occurring in East Africa. The roots have been used for the treatment of diarrhoea by the Kikuyu tribe [1], while the bark has been used as an emetic and the roots as dysentery remedy by the Zulu [2]. When we collected the plant, we were informed by the natives that the bark is eaten by elephants, possibly for medicinal reasons. This fact attracted our attention to the possibility that pharmacologically active components might be contained in the bark. Until then only the seeds of *E. capensis* had been examined and a limonoid, ekebergin [3], was isolated. We have now examined the constituents of *E. capensis* bark and isolated two new acyclic triterpenoids **1** and **2** along with oleanoic acid, 3-epi oleanolic acid and oleanolic acid. This paper deals with the structural elucidation of the two new triterpenoids.

RESULTS AND DISCUSSION

The methanol extract of *E. capensis* bark was suspended in water and extracted with ethylacetate and then *n*-butanol. The ethylacetate fraction was separated by a combination of column chromatography and preparative TLC to yield five compounds: oleanoic acid, 3-epi oleanolic acid, oleanolic acid, and two new acyclic triterpenoids, **1** and **2**. Compound **1** was assigned the molecular formula $C_{30}H_{54}O_4$ (high resolution EI-mass spectrometry). Its IR spectrum suggested the presence of hydroxyl groups (3572, 3464 cm^{-1}) and C=C bonds (1670 cm^{-1}). The ^{13}C NMR spectrum contained 15 peaks, which is just half the number predicted from the HR-mass spectral data. Consequently, a symmetrical structure with 30 carbons was suggested. The ^1H NMR spectral features indicated that **1** structurally resembled squalene (**3**), an acyclic triterpenoid. Thus, the ^1H NMR showed the presence of four olefinic protons (δ 5.14, 5.19), four methyl groups (δ 1.60, 1.62) attached to sp^2 carbons, and six methylene groups (δ 2.02, 2.09) attached to sp^2 carbons. These ^1H NMR signals were almost the same as squalene. Two CH_2 at δ 2.09 and 2.23 (Ha-5,20 and Hb-5,20) and notably two CH_2 at δ 1.42 and 1.58 (Ha-4,21 and Hb-4,21) gave different chemical shift values compared with squalene. Furthermore, two olefinic protons at δ 5.12 (H-3, H-22) in squalene were not found in **1**. Instead, two CH of an α-monosubstitution were present δ 3.35 (H-3, H-22). In squalene, the four terminal methyl groups are attached to sp^2 carbons. In contrast, the corresponding methyl groups of **1** appeared at rather higher field (δ 1.15, 1.20), which suggested that these methyl groups were attached to sp3 carbons. The ^1H NMR data (Table 1) and molecular formula together suggested that the new compound, as depicted in formula **1**, was a derivative of squalene in which two hydroxyl groups were added to each of the terminal double bonds. The ^{13}C NMR and various two dimensional 2D NMR data (H-H, C-H COSY and HMBC shown in Fig. 1) also support this structure.

On conventional acetylation, **1** gave a monoacetate (**1a**) and a diacetate (**1b**). Both were isolated as colourless oils which gave IR absorption bonds for hydroxyl groups and C=O groups. The ^1H NMR

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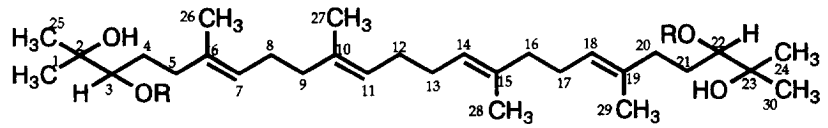
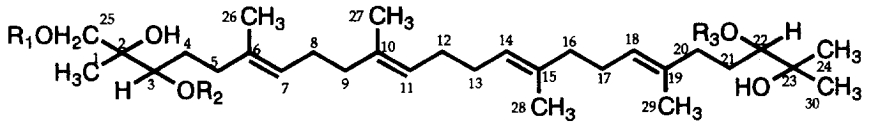
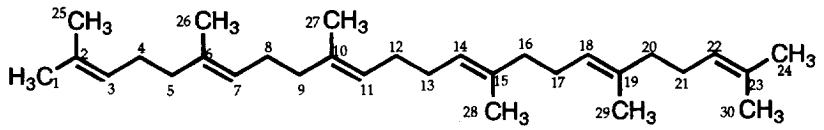


1 R=H
1a R=H, Ac
1b R=Ac



2 R_1=R_2=R_3=H
2a R_1=Ac, R_2=R_3=H
2b R_1=R_2=Ac, R_3=H
2c R_1=R_2=R_3=Ac



3 Squalene

Table 1. Comparison of ^{1}H NMR data of compound **1** and squalene in $CDCl_3$

	1	Squalene
1,24-Me	1.15, 1.20 (each, *s*)	1.68 (*bd*, *J* = 1.0 Hz)
25,30-Me		1.60 (*bs*)
H-3,22	3.35 (*dd*, *J* = 2.0, 10.5 Hz)	5.12 (*m*)
H_2-4,21	1.42, 1.58 (*m*)	2.02 (*m*)
H_2-5, 20	2.09, 2.23 (*m*)	2.09 (*m*)
26,29-Me	1.62 (*bs*)	1.60 (*bs*)
H-7,18	5.19 (*m*)	5.12 (*m*)
H_2-8,17	2.09 (*m*)	2.09 (*m*)
H_2-9,16	2.02 (*m*)	2.02 (*m*)
27,28-Me	1.60 (*bs*)	1.60 (*bs*)
H-11,14	5.14 (*m*)	5.12 (*m*)
H_2-12,13	2.02 (*m*)	2.02 (*m*)

spectrum of **1a** showed signals at δ 2.15 (3H, *s*) due to an alcoholic acetyl group, at δ 3.38 due to a methine proton, and at δ 4.85 due to a methine proton shifted to low-field by acetylation. Because either the C-3 or the C-22 hydroxyl group was acetylated, **1a** was an asymmetric compound. Consequently, the spectrum of **1a** was more complex than that of **1**. The ^{1}H NMR of **1b** showed signals at δ 2.12 (6H, *s*) due to two

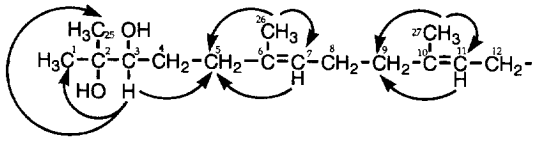


Fig. 1. HMBC data of compound 1.

alcoholic acetyl groups and at δ 4.82 (2H, *dd*) due to methine protons of positions C-3 and C-22 that had been shifted to low-field by acetylation. Because both the C-3 and C-22 hydroxyl groups were acetylated, **1b** was a symmetrical compound. The two tertiary alcoholic groups of **1b** were not acetylated under these conditions.

From these results, we determined the planar structure of **1** to be 2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18-tetracosatetraene. The configuration of the double bonds 6–7, 10–11, 14–15 and 18–19 were identified as all *trans* from the chemical shifts in the ^{1}H and ^{13}C NMR spectra. Thus, as expected the ^{1}H NMR, the signals of methyl protons attached to sp2 carbons appeared at about δ 1.58–1.61 and not δ 1.66–1.69 as would be the case with the *cis* forms. [4, 5]. In the ^{13}C NMR, the corresponding methyl carbons appeared at about δ 15.1–16.0 and not δ 23.4–23.7 as would be the case for the *cis* forms. [5, 6]. The configuration of C-3 and C-22, however, was not determined.

Compound **2** was assigned the molecular formula $C_{30}H_{54}O_5$ (high resolution EI-mass spectrum). The spectral data of **2** were very similar to those of **1**. This suggested that **2** was an acyclic triterpenoid derivative of **1**. As depicted in Fig. 2, most of the signals in **2** corresponding to those found in **1** are split into two, and in addition, a signal at δ 69.24 attributable to the methylene carbon of a hydroxymethyl group is present. These results suggest that **2** is an asymmetrical compound. The situation was also similar in the case of the ^{1}H NMR data. The two degenerated signals at δ 1.15 and δ 1.20 due to four terminal methyl groups present

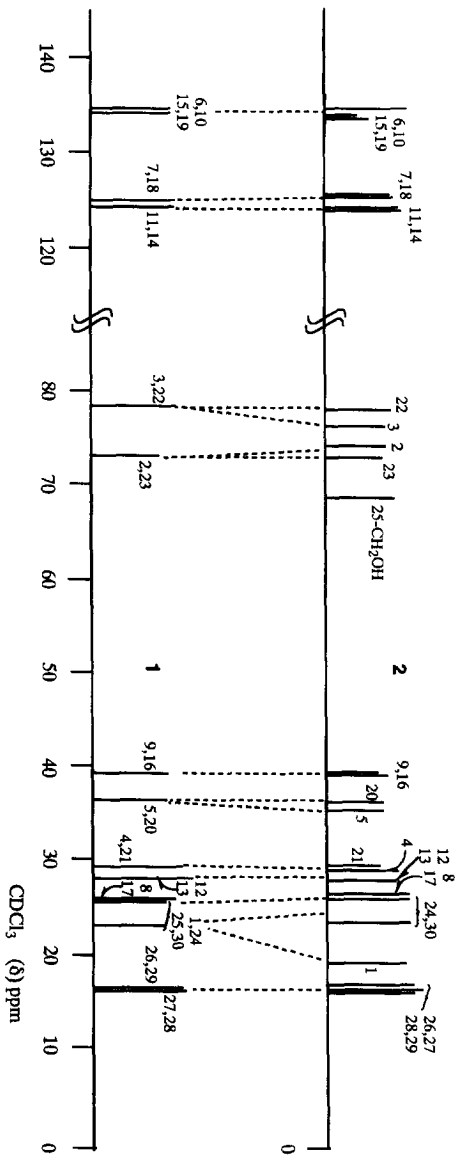


Fig. 2. Line diagram of the ^{13}CNMR spectra of compounds **1** and **2**.

in **1** were replaced by three signals at δ 1.07, 1.15 and 1.20 due to three methyl groups in **2**. Consequently, one terminal methyl group in **1** is missing in **2**. Typical AB type signals at δ 3.50 and δ 3.61 due to methylene protons of a hydroxymethyl group were observed. Based on the high resolution EI-mass spectra and ^{1}H NMR and ^{13}C NMR data, compound **2** has the structure shown in formula 2, where one of the terminal methyl groups of **1** is replaced by a hydroxymethyl group. The HMBC spectral data of **2** (Fig. 3) also support this structure.

On conventional acetylation, **2** gave a monoacetate (**2a**), a diacetate (**2b**) and a triacetate (**2c**). These were all isolated as colourless oils which gave absorption bands for hydroxyl and C=O groups in the IR spectrum. The ^{1}H NMR of **2c** showed signals at δ 2.12 (9H, *s*) due to three alcoholic acetyl groups and signals at δ 4.05 (2H, *bs*, CH_2OAc), 4.83 (1H, *dd*, H-22) and 5.02 (1H, *t*, H-3) that were shifted to low-field on acetylation.

From the above results, we determined the planar

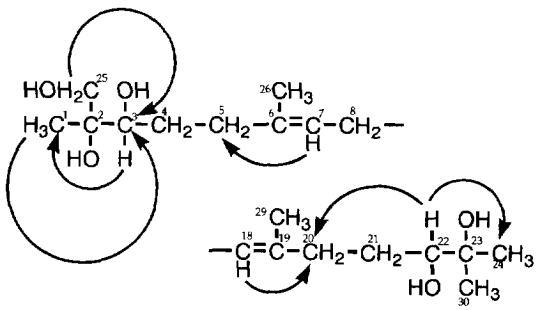


Fig. 3. HMBC data of compound **2**.

structure of **2** to be 2-hydroxymethyl-2,3,23,23-tetrahydroxy-6,10,15,19,23-pentamethyl-6,10,14,18-tetracosatetraene. The configuration of four double bonds were identified as all *trans* by the same reasoning as used in the case of **1**, but the configurations of C-2, C-3 and C-22 were not determined.

Both compounds **1** and **2** are new acyclic triterpenoids in nature.

EXPERIMENTAL

General. ^{1}H NMR: 300 or 500 MHz; ^{13}C NMR: 125 MHz with TMS as int. standard.

Material. The bark of *E. capensis* was collected at Aberdare National Park near Mount Kenya. The plant was identified and authenticated by one of the authors (S.G.M.). The bark was dried in the shade.

Extraction and isolation. The dried bark (515 g) was chopped and extracted with hot MeOH, after defatting with petrol. The MeOH extract (100 g) was suspended in water and extracted with EtOAc (extract 54.2 g) and then *n*-BuOH (extract 8.75 g). Both extracts were positive with $FeCl_3$, vanillin-HCl and *p*-anisaldehyde-H_2SO_4 reagents, which suggested the presence of condensed tannins. On HPLC with photodiode-array detection of the EtOAc fr. several peaks were observed. Two were identified as gallocatechin and epicatechin by comparing their R_t and UV spectra with those of authentic samples.

Other components, in addition to tannins, were detected in this fraction by TLC, therefore, the EtOAc fr. was subjected to silica gel CC, eluting with $CHCl_3$ and $CHCl_3$–MeOH. Each fr. was monitored by TLC using $CHCl_3$–MeOH and *p*-anisaldehyde H_2SO_4 reagent as a developing solvent and a colour reagent, respectively. Oleanoic acid and its 3-epi oleanolic acid were obtained as a mixt., which was sepd by prep. TLC with benzene–Et_2O–HoAc (20:10:1) and the components purified by recrystallization from MeOH. Oleanolic acid was purified by recrystallization from MeOH. Compounds **1** and **2** were purified by prep. TLC with benzene–Et_2O–MeOH (5:5:1). The yields of the aforementioned compounds were 0.30, 0.29, 0.27, 1.19 and 0.80%, respectively. The oleanoic acid, 3-epi oleanolic acid and oleanolic acid, respectively, were identified by comparing their spectral data (especially, ^{1}H, ^{13}C, 2D NMR) with authentic samples and with spectral data in the literature [7, 8].

Compound **1**. Oil, $[\alpha]_D^{22}$ +23° ($CHCl_3$, *c* 6.97). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3572, 3464 (*br.* OH), 1670 (C=C), 1452, 1388, 1162, 1078; EI-MS *m/z* (rel. int.): 478 $[M]^+$ (0.9), 460 $[M-H_2O]^+$ (5.1), 442 $[M-H_2O-H_2O]^+$ (8.3), 153 (100); HREI-MS: found $[M]^+$ 478.4020; $C_{30}H_{54}O_4$, requires 478.4025. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.15, 1.20 (each 6H, *s*, 1, 24, 25, 30-Me), 1.42 (2H, *m*, Ha-4, 21), 1.58 (2H, *m*, Hb-4, 21), 1.60 (6H, *bs*, 27, 28-Me), 1.62 (6H, *bs*, 26, 29-Me), 2.02 (8H, *m*, H_2-9, 12, 13, 16), 2.09 (6H, *m*, Ha-5, 20 and H_2-8, 17), 2.23 (2H, *m*, Hb-5, 20), 3.35 (2H, *dd*, J = 2.0, 10.5 Hz, H-3, 22), 5.14 (2H, *m*, H-11, 14), 5.19 (2H, *m*, H-7, 18); ^{13}C NMR (125 MHz, $CDCl_3$) δ 15.9 (C-27, C-28), 16.0 (C-26, C-29), 23.4, 26.44 (C-1, C-24, C-25 and C-30) 26.6 (C-8, C-17), 28.3 (C-12, C-13), 29.7 (C-4, C-21), 36.8 (C-5, C-20), 39.7 (C-9, C-16), 73.0 (C-2, C-23), 78.3 (C-3, C-22), 124.5 (C-11, C-14), 125.2 (C-7, C-18), 134.9, 135.0 (C-6, C-10, C-15 and C-19). HMBC: Fig. 1.

Acetylation of **1**. Compound **1** (70 mg) was acetylated with Ac_2O in pyridine and the crude acetates were separated by prep. TLC with benzene–Et_2O–MeOH (10:10:1) to yield **1a** (24 mg) and **1b** (34 mg) as oils. **1a**: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3612, 3492 (*br.* OH), 1732 ($OCOCH_3$), 1670, 1454, 1378, 1248; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.17, 1.20, 1.21 (12H, each *s*, $CH_3 \times 4$), 1.61, 1.64 (12H, each *s*, =C–$CH_3 \times 4$), 1.40–1.80 (4H, *m*, H_2-4, 21), 1.85–2.25 (16H, *m*), 2.15 (3H, *s*, $OCOCH_3$), 3.38 (1H, *dd*, J = 2.0, 10.0 Hz, C<u>H</u>-OH), 4.85 (1H, *dd*, J = 3.5, 9.0 Hz, C<u>H</u>-OAc), 5.18 (4H, *m*, H-7, 11, 14, 18). **1b**: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3616, 3492 (*br* OH), 1732 ($OCOCH_3$), 1670, 1454, 1378, 1252; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19, 1.21 (12H, each *s*, $CH_3 \times 4$), 1.61 (12H, each *s*, =C–$CH_3 \times 4$), 1.60–1.83 (4H, *m*, H_2-4, 21), 1.85–2.12 (16H, *m*), 2.12 (6H, *s*, $OCOCH_3 \times 2$), 4.82 (2H, *dd*, J = 3.5, 9.5 Hz, C<u>H</u>-OAc × 2), 5.08 (4H, *m*, H-7, 11, 14, 18).

Compound **2**. Oil, $[\alpha]_D^{22}$ +20° ($CHCl_3$, *c* 2.42). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3464 (*br.* OH), 1675 (C=C), 1454, 1388, 1160, 1076; EIMS *m/z* (rel. int.): 494 $[M]^+$ (0.2), 476 $[M-H_2O]^+$ (1.1), 458 $[M-H_2O-H_2O]^+$ (1.5), 81 (100); HREI-MS: found $[M]^+$ 494.3968: $C_{30}H_{54}O_5$, require 494.3964. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.07 (3H, *s*, 1-Me), 1.15, 1.20 (each 3H, *s*, 24, 30-Me), 1.41 (1H, *m*, Ha-21), 1.53 (3H, *m*, H_2-4 and Hb-21), 1.60 (6H, *bs*, 27, 28-Me), 1.62 (6H, *bs*, 26, 29-Me), 2.02 (8H, *m*, H_2-9, 12, 13, 16), 2.09 (6H, *m*, Ha-5, 20 and H_2-8, 17), 2.23 (2H, *m*, Hb-5, 20), 3.35 (1H, *dd*, J = 1.5, 10.5 Hz, H-22), 3.50 (1H, *d*, J = 11.5 Hz, Ha-25), 3.61 (2H, *bd*, J = 11.0 Hz, Hb-25 and H-3), 5.14 (2H, *m*, H-11, 14), 5.19 (2H, *m*, H-7, 18); ^{13}C NMR (125 MHz, $CDCl_3$) δ 15.9, 15.9, 16.0 (C-26, C-27, C-28, C-29), 19.6 (C-1), 23.3, 26.4 (C-24, C-30), 26.5 (C-8, C-17), 28.2 (C-12, C-13), 29.2 (C-4), 29.7 (C-21), 36.4 (C-5), 36.8 (C-20), 39.6, 39.7 (C-9, C-16), 69.2 (C-25), 73.0 (C-23), 74.1 (C-2), 75.9 (C-3), 78.3 (C-22), 124.5, 124.6 (C-11, C-14), 125.1, 125.2 (C-7, C-18), 134.9, 135.0, 135.0 (C-6, C-10, C-15, C-19); HMBC: Fig. 3.

Acetylation of **2**. Compound **2** (30 mg) was acetylated with Ac_2O in pyridine and the crude acetates were separated by prep. TLC with benzene–Et_2O–MeOH (10:10:1.5) to yield **2a** (5 mg), **2b** (8 mg) and **2c** (13 mg) as oils. **2a**: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500 (*br.* OH), 1736 ($OCOCH_3$), 1456, 1376; ^{1}H NMR (300 MHz, $CDCl_3$). δ 1.16, 1.20 (9H, each *s*, $CH_3 \times 3$), 1.61, 1.62, 1.63 (12H, each *s*, =C–$CH_3 \times 4$), 2.12 (3H, *s*, $OCOCH_3$), 3.39 (1H, *dd*, J = 2.0, 10.0 Hz, H-22), 3.51 (1H, *m*, H-3), 4.02, 4.20 (each 1H, *d*, J = 11.5 Hz, CH_2–OAc), 5.20 (4H, *m*, H-7, 11, 14, 18). **2b**: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3588 (*br.* OH), 1740 ($OCOCH_3$), 1454, 1378; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.17, 1.20, 1.21 (9H, each *s*, $CH_3 \times 3$), 1.62 (12H, *br s*, =C–$CH_3 \times 4$), 2.12 (6H, *s*, $OCOCH_3 \times 2$), 3.37 (1H, *dd*, J = 2.0,

10.0 Hz, H-22), 4.04 (2H, *br s*, CH_2–OAc), 5.01 (1H, *t*, $J = 6.5$ Hz, 3C$\underline{H}$–OAc), 5.18 (4H, *m*, H-7, 11, 14, 18). **2c**: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500 (*br*. OH), 1736 ($OCOCH_3$), 1456, 1378; ^1H NMR (300 MHz, $CDCl_3$) δ 1.21, 1.22 (9H, each *s*, $CH_3 \times 3$), 1.62 (12H, *br s*, $=C-CH_3 \times 4$), 2.12 (9H, *s*, $OCOCH_3 \times 3$), 4.05 (2H, *br s*, CH_2-OAc), 4.83 (1H, *dd*, $J = 3.5$, 9.0 Hz, 22C$\underline{H}$–OAc), 5.02 (1H, *t*, $J = 6.5$ Hz, 3C$\underline{H}$–OAc), 5.19 (4H, *m*, H-7, 11, 14, 18).

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