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FURTHER MINOR TETRANORTRITERPENES FROM *MELIA VOLKENSII*[1]

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ABSTRACT. Two new minor tetranortriterpenes, 2',3'-dihydrosalannin and 1-detigloyl-1-isobutylsalannin, have been isolated from then fruit of *Melia volkensii*. Their structures have been established by spectroscopic and chemical methods. They belong to the ring-C cleaved class of tetranortriterpenes.

INTRODUCTION

Melia is a genus consisting of shrubs, widely spread in Africa and the Indian Ocean Islands. *Melia volkensii* Gürke (Meliaceae) is found in the dry areas of East Africa, it is an attractive deciduous tree often grown for shade or ornament and used by the local medicine men for the treatment of a variety of human disorders. An infusion of the pounded bark is used as a remedy for body aches and pain and is said to be poisonous in an overdose [1].

Extract of *M. volkensii* fruit kernels have been reported to have been antifeedant activity against nymphs and adults of the desert locust, *Schistocerca gregaria* [2]. The same report states that an extract from dry *M. volkensii* fruit exhibited growth inhibiting activity against larvae of the mosquito *Aedes aegypti.* The active fraction was shown to be free of azadirachtin (**1**) and that the active compound may be more patent as an insect growth inhibitor and more lethal to *A. aegypti* larvae than azadirachtin [3]. In an earlier investigation [4,5], we reported the isolation from *M. volkensii* of a new insect antifeedant, volkensin (**2**), together with three new tetranortriterpenes designated as 1-cinnamoyltrichilinin (**3**), 1-tigloyltrichilinin (**4**), 1-acetyltrichilinin (**5**), and the known salannin (**6**) [6] and ohchinin-3-acetate (**7**) [7]. Recently, a report on the structure-activity relationship of salannin (**6**) as an antifeedant against the Colorado Potato Beetle (*Leptinotarsa decemlineata*) indicated that hydrogenation of the tigloyl moiety of salannin (**6**) to give an α-methylbutyryl group resulted in at least a 2-fold increase in its insect antifeedant activity [8]. This interesting observation prompted us to report our results on the isolation and structure determination of 2',3'-dihydrosalannin (**8**), previously reported as a semi-synthetic [8], but was not known as a natural product, together with 1-detigloyl-1-isobutylsalannin (**9**) from the methanolic extract of *M. volkensii* fruit. These two new naturally occurring limonoids belong to the class of tetranortriterpenes bearing a cleaved C-ring. The structure elucidation of **8** and **9** was carried out by spectroscopic techniques and chemical methods.

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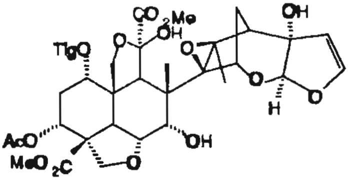
[1] Dedicated to the late Prof. Kilian Mtotomwema, Botany Department, Moi University, who passed away on January 11, 1991.

RESULTS AND DISCUSSION

The ^{1}H NMR spectra of **8** and **9** closely resembled that of salannol (**10**) [9] and salannin (**6**) [6,9], with the major difference associated with ring A. ^{1}H and ^{13}C NMR spectral data (Table 1) suggested the presence of a ß-substituted furan ring, a methyl ester, a quaternary double bond, and an acetoxy group in **8** and **9**. In addition, the spectra of compound **8** and **9** indicated the presence of the following groupings: -$C_1H(O)$-$C_2H(H)$-$C_3H(O)$-, -C_5H-$C_6H(O)$-$C_7H(O)$-, -$C(CH_3)$=C-CH(O)-. Using compound



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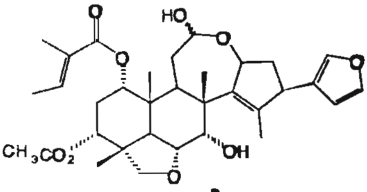

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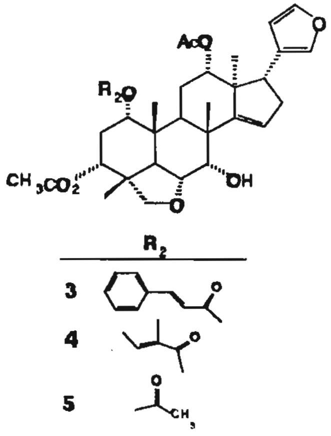
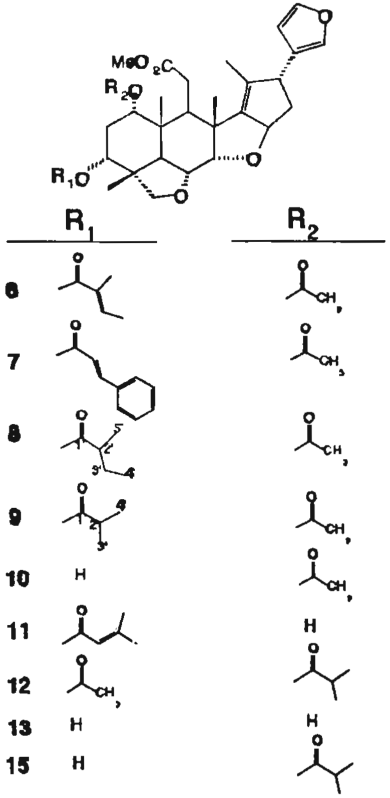
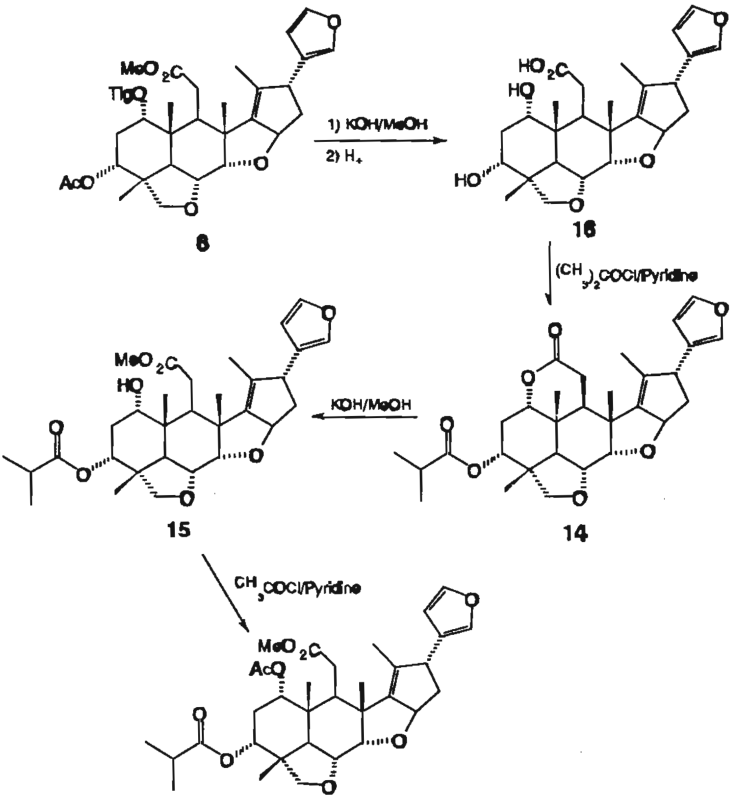




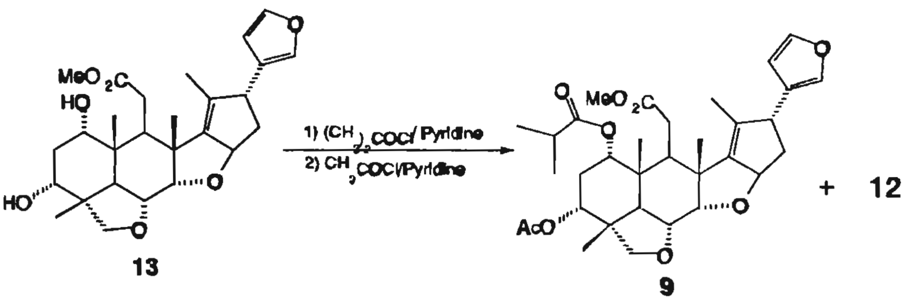
8 as an example, the ^{1}H NMR (200 MHz, $CDCl_3$) is as follows: δ 5.05 (1H, *t*, H-3), 2.35 (1H, *t*, H-2), 5.02 (1H, *t*, H-3) [$J_{H-1,H-2} = J_{H-2,H-3} = 3$ Hz], 2.97 (1H, *d*, H-5), 4.04 (1H, *dd*, H-6), 4.23 (1H, *d*, H-7) [$J_{H-5,H-6} = 12$ Hz and $J_{H-6,H-7} = 3$ Hz], chemical shifts of H-6 and H-7 in **8** and **9** were very similar to those reported for salannin (**6**) [6], salannol (**10**) [9], ohchinin-3-acetate (**7**) [7] and salannolide (**11**) [10]. The oxide link in **8** and **9** are therefore through C-6 and C-28, and C-7 and C-15, respectively. This was fully supported by a two-proton multiplet signal at around δ 3.60 assigned to the C-28 methylene, and a broad triplet at around δ 5.47, showing coupling with the methylene at C-16, attributed to H-15.



Scheme 1. Synthesis of compound **12** from salannin (**6**).

The data given above suggest that the basic skeleton of **8** and **9** constituting rings A, B, C and D was thus identified to that reported for salannin (**6**) and also accounted for all the oxygens in the molecule, The ^{1}H NMR spectra of **8** and **9** however differed from that of salannin (**6**) in the substitution at C-1. The identity of the ester functions of **8** as α-methylbutyrate and acetate, respectively, were indicated by the presence of two diffuse one-proton triplets at around δ 4.9 and 4.8 similar to those reported for salannin (**6**) and related limonoids (each protons at C-1 and C-3 being individually coupled to the methylene protons at C-2). Peaks at δ 2.49 (1H, *tq*, *J* = 7, 7), 1.19 (2H, *d*, *J* = 7.5), 1.52 (1H, *ddq*, *J* = 14, 7, 7), 1.72 (1H, *ddq*, *J* = 14, 7, 7) and 0.92 (3H, *t*, *J* = 7.5) are consistent with the presence of an α-methylbutyryl group in compound **8**. Additional support for the presence of an α-methylbutyrate substituent comes from the mass spectrum; m/z 598 $[M]^+$, 538 $[M\text{-}CH_3COOH]^+$, 496 $[M\text{-}CH_3CH_2CH(CH_3)COOH]^+$. This is further supported by the close similarities in the IR, ^1H NMR and mass spectral data of a synthetic sample of **8** as reported by Yamasaki *et al* [8]. On the other hand peaks at δ 175.2 (C=O), 34.4 (CH), 18.52 (CH_3) in the ^{13}C NMR and δ 1.26 (3H), 1.30 (3H) in the ^1H NMR spectra are consistent with an isobutyryl substituent at C-1 in compound **9**.

In both compounds **8** and **9**, the α-methylbutyryl and isobutyryl groups must be attached at C-1 and the acetyl group at C-3, rather than the reverse, for the following reasons:- In the ^1H NMR spectrum of **8** and **9**, the signal due to the ester group is observed at an unusually high field (δ 3.26 and 3.27, respectively), similar to that observed in salannin (**6**). This is further supported by an unambiguous synthesis of 1-acetyl-3-isobutyl salannin (**12**) (Scheme 1). The synthesis was accomplished via a four step sequence involving hydrolysis of salannin (**6**) to give nimbidic acid (**16**) which was treated with isobutyrylchloride in pyridine to give compound **14** having an isobutyryl substituent at C-3. Compound **14**, was carefully treated with methanolic-KOH to induce selective hydrolysis of the C-1-C-12-lactone followed by an *in situ* methylation of the resulting carboxylic acid to give 3-deacetyl-1-detigloyl-3-isobutylsalannin (**15**). This was then acetylated to give compound **12**. The resonance of the ester group in compound **12** was observed at δ 3.31 consistent with the presence of an acetyl group at C-1. On the other hand, direct reaction of 1-detigloyl-1-deacetyl salannin (**13**) with isobutyryl chloride in pyridine followed by acetylation, yielded compound **12** and a product which was identical to compound **9** (Scheme 2). Unlike **8** and **9**, compound **12** with an acetyl group at C-1 had the ester group resonating at δ 3.31. These results confirm the proposed ring A substitution pattern for compounds **8** and **9**.



Scheme 2. Synthesis of compounds **9** and **12**

In assigning ^{13}C absorptions (Table 1), APT techniques were used to differentiate methyls, methylenes, methines and quaternary carbons. HECTOR and COSY techniques were used to correlate ^{13}C and ^{1}H shift assignments. In addition, ^{13}C and ^{1}H NMR shifts reported by Kraus *et al.* for **6** [9] and related limonoids were useful in making assignments for **8** and **9**. These compounds, like other related limonoids may display interesting bioactivities.

Table 1. ^{13}C NMR data for compounds **8** and **9** in $CDCl_3$ at 50.3 MHz.

Carbon No.	**8**	**9**
1	72.69	72.62
2	28.89	30.28
3	71.41	71.33
4	42.82	42.74
5	39.15	39.83
6	70.64	70.64
7	85.67	85.57
8	48.84	48.84
9	39.14	39.12
10	40.42	40.33
11	30.36	30.28
12	172.86	172.72
13	134.83	134.69
14	146.51	146.38
15	87.90	87.84
16	41.25	41.20
17	49.51	49.46
18	15.56	15.54
19	16.92	16.98
20	127.21	127.07
21	138.89	138.78
22	110.76	110.67
23	142.87	142.76
28	77.93	77.80
29	19.63	19.52
30	12.94	12.93
1'	175.93	176.00
2'	54.07	53.49
3'	26.14	28.91
4'	11.85	28.18
5'	29.63	---
$CH_3\underline{C}O$	171.11	170.58
$\underline{C}H_3CO$	21.48	20.96
$COO\underline{C}H_3$	51.11	51.32

EXPERIMENTAL

General: Melting points were determined on a Fisher hot stage apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 283 spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained on a Varian XL-200. Low resolution mass spectral data were recorded on an HP-5985 mass spectrometer operating at 70 ev. High resolution mass spectra were obtained on a VG-70E system.

Extraction: Ripening fruit of *M. volkensii* was collected in Tsavo Park, Kenya. A voucher specimen of the leaves is deposited in the University of Nairobi herbarium. Crushed whole fruit (7.9 kg) was allowed to stand 2 weeks in 8 L of MeOH. The extract was decanted, and the residual pulp was similarly extracted a second time. The combined extracts were evaporated under vacuum, and the residue was partitioned between $CHCl_3$ and H_2O. After drying over Na_2SO_4, the $CHCl_3$ layer was evaporated under vacuum to yield 64 g of a thick brown oil.

Isolation and identification: Column chromatography was performed on 11.6 g of the oil using silica gel (70-130 mesh, Merck) eluted with hexane/acetone (3:1) and the separation was monitored by TLC (silica gel, 4:10 acetone/hexane). The two limonoids eluted in the order 2',3'-dihydrosalannin (**8**) (110 mg) and 1-detigloyl-1-isobutylsalannin (**9**, 100 mg), respectively.

2',3'-dihydrosalannin (**8**): 110 mg of crude **8** was purified further by preparative TLC (silica gel, Merck) eluted with 30% methyl ethyl ketone in toluene, to give pure 2',3'-dihydrosalannin (**8**) (80 mg, 0.06% isolated yield from the whole fruit) as a colourless thick oil which failed to crystallize. The compound was however homogenous on five different solvent systems. IR (thin film) cm^{-1}: 1715 (C=O), 1220, 1130, 1030 (C-O), 3110, 850 (furan); MS m/z: 598 $[M]^+$. HRMS m/z: 598.3170 $[M]^+$ (calc. for $C_{34}H_{46}O_9$: 598.3143); ^1H NMR (200 MHz, $CDCl_3$): δ 0.95 (6H, *t*, *J* = 7.5 Hz, 19-Me, 4'-Me), 1.25 (3H, *s*, 29-Me), 1.28-1.31 (3H, *m*, 5'-Me), 1.31 (3H, *s*, 30-Me), 1.65 (3H, *m*, 18-Me), 2.08 (3H, *s*, OAc), 2.63-2.76 (2H, *m*, H-9, H-5), 3.26 (3H, *s*, OMe), 3.60 (3H, *m*, H-17, H-28), 3.97 (1H, *dd*, *J* = 12.4, 3.2 Hz, H-6), 4.15 (1H, *d*, *J* = 3.2 Hz, H-7), 4.88 (1H, *t*, *J* = 3.7 Hz, H-1), 4.97 (1H, *t*, *J* = 3.7 Hz, H-3), 5.47 (1H, *m*, H-15), 6.33 (1H, *m*, H-22), 7.28-7.33 (2H, *m*, H-21, H-23); ^{13}C NMR: see Table 1.

1-detigloyl-1-isobutylsalannin (**9**): 100 mg of crude (**9**) was purified further by preparative TLC (silica gel, Merck) and eluted with 30% methyl ethyl ketone in toluene, to give pure 1-detigloyl-1-isobutylsalannin (**9**) (60 mg, 0.05% isolated yield from the whole fruit) as a colourless thick oil which failed to crystallize. This compound, like the previous one was homogenous on TLC using five different solvent systems. IR (thin film) cm^{-1}: 1715 (C=O), 1220, 1130, 1030 (C-O), 3110, 850 (furan); MS m/z: 584 $[M]^+$; HRMS m/z: 584.2982 $[M]^+$ (calc. for $C_{33}H_{44}O_9$: 584.2986); ^1H NMR (200 MHz, $CDCl_3$): δ 0.95 (3H, *s*, 19-Me), 1.21 (3H, *s*, 29-Me), 1.31 (3H, *s*, 30-Me), 1.65 (3H, *m*, 18-Me), 1.26-1.31 (6H, *m*, 3'-Me, 4'-Me), 2.07 (3H, *s*, OAc), 2.63-2.78 (2H, *m*, H-5, H-9), 3.27 (3H, *s*, OMe), 3.60 (3H, *m*, H-28, H-17), 3.98 (1H, *t*, *J* = 12.4, 3.2 Hz, H-6), 4.15 (1H, *d*, *J* = 3.2 Hz, H-7), 4.85 (1H, *t*, *J* = 3.7 Hz, H-1), 4.97 (1H, *t*, *J* = 3.7 Hz, H-3), 5.47 (1H, *m*, H-15), 6.33 (1H, *m*, H-22), 7.28-7.34 (2H, *m*, H-21, H-23); ^{13}C NMR: see Table 1.

Nimbidic acid. (**16**): Salannin (**6**, 400 mg) was dissolved in 5% methanolic KOH and heated under reflux overnight. The solution was then diluted with water, acidified with 10% HCl and extracted with ethyl acetate (20 ml x 3), to give crude nimbidic acid. The crude product was purified by preparative TLC using acetone/hexane (1:1) containing a few drops of acetic acid. The resulting white solid (260 mg, 84% yield) had identical melting point, ^1H NMR, MS and IR spectra to those reported [11] for nimbidic acid (**16**).

3-isobutylnimbidic acid-1 (12)-lactone (**14**): Nimbidic acid (**16**, 200 mg) was dissolved in pyridine (15 ml) and treated with an excess of isobutyryl chloride. The mixture was left stirring at room temperature for 24 hrs. The pyridine was then evaporated with nitrogen and the resulting crude product purified by preparative TLC (silica gel, Merck) using 30% acetone in hexane as the eluting solvent to give isobutylnimbidic acid-1(12)-

lactone (**14**) as a thick colourless oil (184 mg, 80% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.13 (*s*), 1.17 (*s*), 1.23 (*s*), 1.30 (*s*), 1.32 (*s*) [CH_3 x 5], 1.76 (3H, *m*, 18-Me), 2.64 (1H, *d*, H-5), 3.59 (1H, *d*, H-28), 3.72 (1H, *br.d*, H-17), 3.81 (1H, *d*, H-28), 4.08 (1H, *dd*, H-6), 4.31 (1H, *d*, H-7), 4.41 (1H, *dd*, H-1), 4.83 (1H, *dd*, H-3), 5.38 (1H, *m*, H-15), 6.14 (1H, *m*, H-22), 7.16-7.36 (2H, *m*, H-21, H-23).

3-deacetyl-1-detigloyl-3-isobutylsalannin (**15**): Compound (**14**, 100 mg) was dissolved in 10 ml of MeOH and 1 ml of 1 N KOH added. The mixture was stirred for 30 min. and then acidified with 10% HCl. The MeOH was then removed under vacuum and the resulting solution extracted with $CHCl_3$ (5 ml x 3). The combined $CHCl_3$ extracts were dried with anhydrous Na_2SO_4, concentrated and purified by preparative TLC (silica gel, Merck) using 30% acetone in hexane give a thick colourless oil (90 mg, 85% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.93-1.23 (19-Me, 29-Me, 30-Me, 4'-Me, 3'-Me), 1.72 (3H, *m*, 18-Me), 3.51 (3H, *s*, OMe), 3.56 (2H, *br.s*, H-28), 3.66 (1H, *br.d*, H-17), 3.99 (1H, *dd*, H-6), 4.19 (1H, *d*, H-7), 5.03 (1H, *t*, H-3), 5.50 (1H, *m*, H-15), 6.33 (1H, *m*, H-22), 7.27, 7.33 (2H, *m*, H-21, H-23).

3-deacetyl-1-detigloyl-3-isobutyl-1-acetylsalannin (**12**): Compound (**15**, 50 mg) was dissolved in 2 ml of pyridine and an excess of acetyl chloride added. The mixture was stirred at room temperature for 24 hrs. The pyridine was then evaporated with nitrogen and the resulting brownish crude product purified by preparative TLC (silica gel, Merck) using 30% acetone in hexane as eluting solvent to give compound **12** as a thick colourless oil (40 mg, 74% yield). ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.95-1.30 (19-Me, 29-Me, 30-Me, 4'-Me, 3'-Me), 1.65 (3H, *m*, 18-Me), 2.33 (3H, *s*, OAc), 3.31 (3H, *s*, OMe), 3.58 (3H, *m*, H-28, H-17), 3.97 (1H, *dd*, H-6), 4.15 (1H, *d*, H-7), 4.94 (2H, *m*, H-1, H-3), 5.44 (1H, *m*, H-15), 6.33 (1H, *m*, H-22), 7.28, 7.34 (2H, *m*, H-21, H-23).

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