# TIRUCALLANE AND OLEANANE TRITERPENES FROM THE RESIN OF AUCOUMEA KLAINEANA *

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Abstract—The oleoresin of *Aucoumea klaineana* yielded nine triterpenes, six based on the tirucallane and three on the oleanane series. Of the tirucallane derivatives, four appear to be novel natural products, although two of these have previously been synthesized. They are tirucalla-7,24-dien-3,21-dione-21,23-oxide (flindissone lactone), 3α-hydroxy-21-oxotirucalla-7,24-dien-21,23-oxide (flindissol lactone), 21-hydroxy-3-oxotirucalla-7,24-dien-21,23-oxide (flindissone as a mixture of the C-21 epimers) and 22ξ-hydroxytirucalla-7,24-dien-3,23-dione. The other five triterpenes are flindissol, 3α-hydroxytirucalla-7,24-dien-21-oic acid, β-amyrin, β-amyrenone and maniladiol (3β,16β-dihydroxyolean-12-ene).
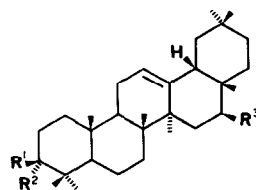
## INTRODUCTION

*Aucoumea klaineana* Pierre [syn. *Dacryodes klaineana* (Pierre) H. J. Lam] is a tree of the humid tropical forests of West and Central Africa [2]. Previous studies on the oleoresin have yielded monoterpenes [1, 3] and a number of triterpenes based on ursane and turicallane [4]. In this paper we report the results of a further examination of the resin and the isolation of an extensive range of triterpenes including four that appear to be novel.

## RESULTS AND DISCUSSION

Following steam distillation to remove monoterpenes [1], the resin was extracted with pentane. Column chromatography of an aliquot of the pentane-soluble fraction over silica gel yielded seven compounds. A second aliquot of pentane soluble material was extracted into methanol and the soluble fractions, subjected to the same chromatographic procedures to yield two further compounds.
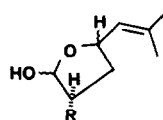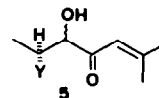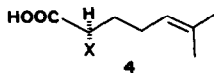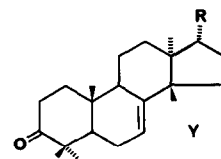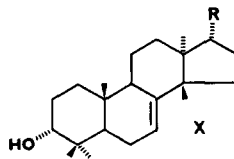
Three of the isolated compounds were pentacyclic triterpenes of the oleanane series. Two were characterized as β-amyrin (1) and β-amyrenone (2) by interconversion and comparison with published data and authentic samples. The third analysed for $C_{30}H_{50}O_2$ and proved to be the rare maniladiol (3). The recent literature has been confused regarding the assignment of the 16-hydroxy substituent of 3 to either the equatorial (β) [5] or axial (α) [6] configuration. The large *J* value of 11.4 Hz for H-16
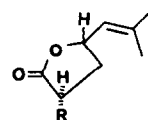


1    $R^1$=OH    $R^2$=$R^3$=H

2    $R^1$,$R^2$=O    $R^3$=H

3    $R^1$=$R^2$=OH    $R^3$=H



4



5



6    R=X

7    R=Y

8    R=X

9    R=Y

clearly establishes an axial ($\alpha$) configuration for this proton and this is supported by an NOE experiment in which irradiation of Me-27 ($\delta 1.19$) causes enhancement of both H-16$\alpha$ (5.4%) and H-19$\alpha$ (4.8%) protons.

The remaining six compounds were all tetracyclic triterpenes and gave $^1$H NMR signals indicative of 3-oxo or 3$\alpha$-hydroxy substituents and an olefinic proton at C-7 [4, 7, 8]. The most polar was characterized as the acid 4 which has previously been isolated from this species [4] and from elemi resin (*Canarium* sp.—also Burseraceae) [9]. Its high-field $^1$H NMR (Table 1) and $^{13}$C NMR spectra (Table 2) are recorded here for the first time.

A second compound analysed for $C_{30}H_{46}O_3$ with the IR spectrum indicating an OH and two C=O moieties. The EIMS gave significant fragments for $m/z$ 114 $[C_6H_{10}O_2]^+$ and 371 $[C_{25}H_{39}O_2]^+$ requiring the presence of two oxygen atoms in the side-chain. In the $^1$H NMR spectrum, the presence of a 3-oxo function was confirmed by the *ddd* at $\delta 2.74$ for the H-2 axial proton. The H-7 olefinic proton occured as a *dt* at $\delta 5.32$ (Table 1) while the H-24 olefinic proton resonated at 6.07, about 1 ppm more deshielded than in 4, suggesting that it was located $\alpha$ to a carbonyl. Furthermore, H-24 exhibited three long-range couplings, to the C-26 and C-27 methyls and to an oxymethine proton (H-22) resonating at $\delta 4.16$. The latter revealed only two couplings, the second being

to H-20, so allowing formulation of the side-chain as $-CH(Me)CH(OH)COCH=C(Me)_2$. On this basis, the compound is assigned structure 5 which appears to be novel. However, it should be noted that the evidence presented does not rule out the C-20 euphane epimer although the apparent occurrence of the tirucallane nucleus for all the other tetracyclic triterpenes isolated makes this highly unlikely.

The $^1$H NMR spectra of the other four compounds all revealed an oxymethine resonance at *ca* $\delta 5.0$ as a multiplet coupling to the olefinic H-24 and showed a major ion in the EIMS for $m/z$ 55 $[C_4H_7]^+$. One was identified as flindissol (6), which has previously been isolated from several species of the Rutaceae [10, 11]. A second differed from 6 only in oxidation of the 3$\alpha$-hydroxyl function to the corresponding 3-oxo moiety (Table 1) and so must be flindissone (7), which has been synthesized [12] but is a new natural product. A characteristic of both 6 and 7 is the appearance of signals in the $^1$H NMR spectrum for both C-21 epimers, in a ratio of *ca* 2:1 (Table 1).

The remaining two compounds were similar to 6 and 7 in most respects but exhibited a strong fragment for $m/z$ 139 $[C_8H_{11}O_2]^+$ and a band for a $\gamma$-lactone in the IR spectrum, while in the $^1$H NMR spectrum no resonance was seen for H-21. These observations were all suggestive of the 21-oxo type of flindissol derivatives and other

Table 1. $^1$H NMR chemical shifts for selected protons in compounds 4–9

| H | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|
| 1$_{eq}$ | | 1.83 *m* | | | | |
| 1$_{ax}$ | | 1.45 *dt* (9.0, 3.4) | | | | |
| 2$_{ax}$ | | 2.74 *d* (14.6, 5.4) | | 2.70 *dt* (14.0, 5.0) | | 2.77 *dt* (14.6, 5.6) |
| 3$_{eq}$ | 3.65 *t* (1.5) | | 3.44 *t* (1.5) | | 3.44 *t* (2.5) | |
| 7 | 5.38 *br d* (3.0) | 5.32 *dt* (3.5, 3.0) | 5.26 *m* | 5.30 *m* | 5.24 *dt* (3.7, 3.0) | 5.32 *br d* (3.1) |
| 20 | 2.60 *dt* (11.0, 3.3) | 1.83 *m* | | | 2.67 *m* | 2.74 *m* |
| 22 | | 4.16 *t* (1.5) | | | 2.73 *m*/ 1.82 *m* | |
| 23 | | | 4.78 *m*/ 4.82 *m* | 4.70 *m*/ 4.81 *m* | 5.00 *ddd* (14.3, 8.6, 5.7) | 5.07 *m* |
| 24 | 5.32 *dqq* (7.2, 1.0) | 6.07 *dqq* (1.3, 1.1) | 5.11 *br d* (8.4) 5.24 *m*\* | 5.13 *dqq* (8.4, 1.3) 5.24 *m*\* | 5.18 *dqq* (7.2, 1.4) | 5.20 *br d* (8.6) |
| Me-26/ | 1.62 *d* (0.8) | 1.96 *d* (1.1) | 1.71/ 1.70\* | 1.73/ 1.70\* | 1.71 *d* (1.3) | 1.73 *d* (1.1) |
| Me-27 | 1.67 *d* (0.8) | 2.01 *d* (1.0) | 1.68/ 1.68\* | 1.71/ 1.68\* | 1.75 *d* (1.3) | 1.77 *d* (1.1) |
| Me/H-21 | | 0.63 *d* (6.6) | 5.20–5.30 *m* | 5.20–5.30 *m* | | |
| Me-18/ | 0.84 *s* | 0.85 *s* | 0.75 *s* | 0.85 *s*/0.90\* | 0.76 *s* | 0.82 *s* |
| Me-19/ | 0.97 *s* | 1.00 *s* | 0.89 *s* | 1.01 *s* | 0.80 *s* | 1.02 *s* |
| Me-28/ | 1.09 *s* | 1.04 *s* | 0.89 *s* | 1.01 *s* | 0.89 *s* | 1.03 *s* |
| Me-29/ | 1.12 *s* | 1.06 *s* | 0.90 *s* | 1.05 *s* | 0.91 *s* | 1.07 *s* |
| Me-30 | 1.14 *s* | 1.10 *s* | 0.95 *s* | 1.01 *s* | 1.01 *s* | 1.12 *s* |

All run in CDCl$_3$ except for 4 which was run in C$_5$D$_5$N.
Spectra of 6, 7 and 9 run at 250 MHz, the remainder at 360 MHz.
\* Signals of the minor epimer.

Table 2. $^{13}$C NMR chemical shifts for compounds **4**, **5** and **8** (assignments made according to refs [8, 15])

| C | 4 | 5 | 8 |
|---|---|---|---|
| 1 | 32.0 * | 38.4 | 31.1 * |
| 2 | 26.4 | 34.8 | 25.3 |
| 3 | 74.2 | 216.8 | 76.1 |
| 4 | 36.8 | 47.8 | 37.3 |
| 5 | 43.8 | 52.3 | 44.5 |
| 6 | 23.3 | 24.3 | 23.8 ** |
| 7 | 117.6 | 117.8 | 118.1 |
| 8 | 145.2 | 145.7 | 145.5 |
| 9 | 49.1 ** | 48.4 * | 48.5 |
| 10 | 34.1 | 34.9 | 33.7 |
| 11 | 16.7 | 18.1 | 17.2 |
| 12 | 30.8 * | 33.4 | 34.7 |
| 13 | 42.8 | 43.2 | 43.6 |
| 14 | 50.3 | 51.3 | 50.4 |
| 15 | 32.8 * | 33.9 | 34.5 |
| 16 | 29.7 | 27.9 | 31.0 * |
| 17 | 47.9 ** | 49.0 * | 41.2 |
| 18 | 12.3 | 12.7 | 12.8 |
| 19 | 21.0 *** | 21.5 ** | 21.6 |
| 20 | 47.5 ** | 39.4 | 46.7 |
| 21 | 177.2 | 11.9 | 178.5 |
| 22 | 25.6 **** | 78.6 | 23.6 ** |
| 23 | 25.4 **** | 201.2 | 75.0 |
| 24 | 123.8 | 119.1 | 123.1 |
| 25 | 130.6 | 159.4 | 139.3 |
| 26/27 | 24.6/16.6 | 24.4/21.3 ** | 25.6/18.3 |
| 28 | 27.5 | 28.0 *** | 27.3 *** |
| 29 | 21.8 *** | 21.8 ** | 23.3 |
| 30 | 26.3 | 27.5 *** | 27.6 *** |

All run in CDCl$_3$ except for **4** which was run in C$_5$D$_5$N.
All spectra run at 90.56 MHz.

* For chemical shifts under any compound with an equal number of (*) assignments may be reversed.

spectral data suggested that they represented the 3α-hydroxy compound flindissol lactone (**8**) and the 3-oxo compound flindissone lactone (**9**), respectively. The relationship was confirmed by oxidation of **7** which gave a product identical in all respects to **9**. Both **8** and **9** appear to be novel natural products, although **9** has previously been obtained from **6** [10–12].

The isolation of several tirucallane-type triterpenes with C-21 oxidised is significant chemotaxonomic evidence in favour of the link between the Burseraceae and its supposed allies in the Rutales (Rutaceae, Meliaceae, Simaroubaceae). The only previous example in which C-21 oxidation and cyclisation have been recorded in the Burseraceae are the sapelins of *Bursera klugii* (21,24 or 21,25 oxides) [13]. The 21,23 oxides represent postulated precursors of the furanoid ring system which is a feature of the tetranortriterpenoids of the Rutaceae and Meliaceae [14].

## EXPERIMENTAL

*Plant material.* A sample of *okoume* resin was supplied by Mr C. Wilks, Libreville, Gabon.

*Extraction and isolation of triterpenes.* The resin (83 g) was steam distilled to give an oil (5 g) [1] and the residue dried and extracted into pentane. The extract was filtered and concd to give a residue (70 g), of which 20 g was subjected to CC over silica gel. Elution with petrol (bp 60–80°) containing increasing amounts of EtOAc gave, in order of elution, **2** (200 mg), **1** (1.2 g), **5** (18 mg), **3** (51 mg), **7** (500 mg), **6** (237 mg) and **4** (234 mg). The remaining 50 g of residue was dissolved in hot MeOH and on standing gave a ppt. (20 g) which was filtered off. The filtrate was concd and subjected to CC using the same system as above to give **9** (13 mg) and **8** (246 mg).

*β-Amyrin* (**1**). Found: [M]$^+$ 426.3857; C$_{30}$H$_{50}$O requires 426.3861. Identical in all respects (IR, mmp, OR, $^1$H NMR) with authentic material.

*Amyrenone* (**2**). Found: [M]$^+$ 424.3744; C$_{30}$H$_{48}$O requires 424.3705. Identical in all respects (IR, OR, $^1$H NMR) with authentic material.

*Maniladiol* (**3**). Clusters from petrol–EtOAc, mp 206–209° (Lit. [5] 212–214°); [α]$_D$ + 68° (CHCl$_3$; c 0.2) (Lit. [5] +68°). Found: [M]$^+$ 442.3818; C$_{30}$H$_{50}$O$_2$ requires 442.3811. $^1$H NMR (360 MHz, CDCl$_3$): δ0.76, 0.77, 0.87, 0.88, 0.91, 0.97, 0.98, 1.19 (8 × s, 8 × Me), 2.13 (1H, dd, J = 14.0, 4.6 Hz, H-18), 3.19 (1H, dd, J = 10.7, 4.6 Hz, H-3), 4.16 (1H, dd, J = 11.4, 4.9 Hz, H-16), 5.23 (1H, t, J = 3.6 Hz, H-12). $^{13}$C NMR (90.56 MHz, CDCl$_3$): s at 143.4 (C-13), 43.7 (C-14), 39.8 (C-8), 38.7 (C-4), 37.2 (C-10), 36.8 (C-17), 30.8 (C-20), d at 122.2 (C-12), 78.8 (C-3), 65.9 (C-16), 55.1 (C-5), 49.0 (C-18), 46.8 (C-9), t at 46.5 (C-19), 38.5 (C-1), 35.5 (C-15), 34.1 (C-21), 32.6 (C-7), 30.5 (C-22), 27.1 (C-2), 23.4 (C-11), 18.2 (C-6), q at 33.1 (C-29), 28.0 (C-23), 27.0 (C-27), 23.8 (C-30), 21.3 (C-28), 16.7 (C-26), 15.4, 15.3 (C-24, C-25). EIMS m/z (rel. int.): 442 [M]$^+$ (15), 234 (100), 216 (18), 207 (15), 190 (10), 95 (13), 81 (12), 69 (21).

*3α-Hydroxytirucalla-7,24-dien-21-oic-acid* (**4**). Plates from CHCl$_3$, mp 188–190° (Lit. [4] 214°), [α]$_D$ −40° (CHCl$_3$; c 1.04) (Lit. [9] −34° for methyl ester). Found: [M]$^+$ 456.3608; C$_{30}$H$_{48}$O$_3$ requires 456.3603. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3380, 1700. $^1$H NMR—see Table 1. $^{13}$C NMR—see Table 2. EIMS m/z (rel. int.): 456 [M]$^+$ (35), 441 (71), 423 (100), 327 (6), 281 (13), 187 (25), 175 (11), 159 (12), 147 (17), 135 (20), 133 (24), 119 (25), 107 (25), 105 (32), 95 (42), 83 (23), 81 (27), 69 (42), 55 (58).

*22ξ-Hydroxytirucalla-7,24-dien-3,23-dione* (**5**). Prisms from petrol–EtOAc, mp 186–192°, [α]$_D$ +37° (CHCl$_3$; c 0.30). Found: [M]$^+$ 454.3450; C$_{30}$H$_{46}$O$_3$ requires 456.3447. UV $\lambda_{max}^{EtOH}$ nm (log ε): 238 (4.39). IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3420, 1705, 1670. $^1$H NMR—see Table 1. $^{13}$C NMR—see Table 2. EIMS m/z (rel. int.): 454 [M]$^+$ (15), 439 (14), 421 (2), 371 (13), 355 (19), 341 (44), 325 (44), 313 (6), 297 (6), 125 (10), 123 (12), 114 (76), 85 (18), 83 (100), 55 (39).

*Flindissol* (**6**). Found [M]$^+$ 456.3623; C$_{30}$H$_{48}$O$_3$ required 456.3603. $^1$H NMR—see Table 1. Identical (TLC, IR) with an authentic sample [11].

*Flindissone* (**7**). Plates from petrol–EtOAc, mp 127–130°. Found: [M]$^+$ 454.3480; C$_{30}$H$_{46}$O$_3$ requires 454.3447. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3380, 1720. $^1$H NMR—see Table 1. EIMS m/z (rel. int.): 454 [M]$^+$ (79), 439 (25), 436 (53), 421 (32), 313 (17), 150 (62), 107 (35), 105 (42), 95 (100), 55 (63). Compound **7** (150 mg) in pyridine (1.5 ml) was treated with Jones' Reagent and after normal work-up followed by purification over a silica gel column gave **9** (10 mg).

*Flindissol lactone* (**8**). Plates from petrol–EtOAc, mp 229–234°, [α]$_D$ −50° (CHCl$_3$; c 1.18). Found: [M]$^+$ 454.3434; C$_{30}$H$_{46}$O$_3$ requires 454.3447. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3560, 1750, 1680. $^1$H NMR—see Table 1. $^{13}$C NMR—see Table 2. EIMS m/z (rel. int.): 454 [M]$^+$ (22), 439 (63), 426 (15), 421 (31), 207 (36), 189 (54), 187 (43), 173 (28), 145 (28), 139 (12), 135 (28), 133 (42), 121 (45), 111 (5), 109 (51), 95 (100), 91 (51), 81 (70), 55 (96).

*Flindissone lactone* (**9**). Clusters from petrol–EtOAc, mp 193–195° (Lit. [12] 193–195°), [α]$_D$ −68° (CHCl$_3$;

c 0.21) (Lit. [10] −81°). Found: $[M]^+$ 452.3270; $C_{30}H_{44}O_3$ requires 452.3298. $^1H$ NMR—see Table 1. IR, EIMS, mmp, TLC were all identical with an authentic sample [11].

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