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Triterpenoid derivatives from *Canarium schweinfurthii* Engl. (Burseraceae)

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Abstract
The chemical constituents of a resin of the West African *Canarium schweinfurthii* Engl. led to the characterization of five tirucallane triterpenoid acids (1-5) including a new 3α-acetoxy-28-hydroxytirucalla-8,24-dien-21-oic acid (1) and two known pentacyclic triterpenoids, α-amyrin and β-amyrin were isolated. The structures of the compounds were determined after the analysis of their NMR spectroscopic data including 2D NMR spectra and by comparison of the NMR spectroscopic data reported in the literature. The ^{1}H and ^{13}C NMR data for the new 3α-acetoxy-28-hydroxytirucalla-8,24-dien-21-oic acid (1) are reported here. Compounds 1 and 5 were tested against the NCI panel of human tumour cell lines at a single dose of 10 μM. Compound 1 was the most active showing a 42% growth inhibition against the leukaemia HL-60 (TB) line.

Keywords: *Canarium schweinfurthii*, 3α-acetoxy-28-hydroxytirucalla-8,24-dien-21-oic acid, tirucallane triterpenoids, leukaemia HL-60 (TB) cell line

Introduction
Canarium schweinfurthii Engl. (Burseraceae), a tree with a cylindrical bole, is native to tropical West Africa and grows to about 50 m high [1]. *C. schweinfurthii* is mainly found in equatorial forest regions from Cameroon, Central African Republic, Gabon to Congo [2] and it is used in folk medicine for a variety of ailments including malaria, fever, diarrhea [3, 4], postpartum pain, rheumatism and as a stimulant and emollient. [1] Various parts of *C. schweinfurthii* have been reported for various biological activities such as analgesic, anti-inflammatory, antimicrobial and antioxidant [1]. Several compounds have been reported from this plant which include α-amyrin, oleanolic acid, α-amyrenone, erythodiol, 3-oxotirucalla-8, 24-dien-21-oic acid (β-elemonic acid), 3α-hydroxytirucalla-7,24-dien-21-oic acid (α-elemolic acid or epielemadienolic acid), 3α-hydroxytirucalla-8,24-dien-21-oic acid and 3α-acetoxytirucalla-7,24-dien-21-oic acid [5, 6], 3β-fluorotirucalla-7, 24-dien-21-oic acid [5], elemadienediol [7], schweinfurthinol [8] and canarene [1]. *Canarium* L. belongs to the family of Burseraceae Kunth. in the order Sapindales Juss. exBercht. & J. Pearl. The approximate number of species of *Canarium* is under revision where interestingly all but one of the remaining ca. 115 species occurs. Approximately 3 or 4 species occur in tropical Africa [10, 12, 13]. *Canarium* is regarded as a useful resin resource and famously known for the elemi resin, present in the Philippine's *Canarium luzonicum*, Australian *Canarium muelleri*, *Canarium benghalense* [9] and the West African *Canariun schweinfurthii* [1, 5, 9]. Tiricullane triterpenoid acids constitute the main compounds present in the elemi resin and other extracts of the following *Canarium* species: *C. indicum (*syn. *C. commune*), *C. asperum* [14], *C. ovatum* and *C. boivinii* [15].

Materials and Methods
General experimental procedures
IR spectra were recorded using a Perkin-Elmer (2000 FTIR) spectrophotometer using KBr windows. ^{1}H, ^{13}C and 2D NMR spectra were recorded on a Bruker AVANCE III NMR spectrometer, operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C, using standard experiments from the Bruker pulse programs library. Chemical shifts are reported in ppm (δ) referencing the solvent signal ($CDCl_3$ or CD_3OD) as internal standard respect to TMS (0 ppm), and coupling constants (*J*) are measured in Hz. HR-ESIMS was performed on a Bruker MicroToF Mass Spectrometer, using an Agilent 1100 HPLC to introduce samples. Gravity column chromatography was performed using silica gel (Merck 230–400 mesh) packed 1 or

4 cm diameter columns. Compounds were visualized under UV light at 365 nm, followed by spraying with 1% vanillin-H_2SO_4 spray reagent and heating.

Plant material

The resin of *C. schweinfurthii* was collected from Yaounde, Cameroon, in May 2013 and identified by Mr. Victor Nana, the botanist at the Cameroon National Herbarium. A voucher specimen (HNC 25918) was deposited at the Cameroon National Herbarium.

Extraction and isolation

The resin (130 g) was extracted with dichloromethane (2 l) at room temperature. The extract (100 g) was subjected to column chromatography (CC) over silica gel (300 g, column: 100 x 8 cm) and eluted with hexane followed by hexane/EtOAc mixtures with increasing proportions of EtOAc to obtain six fractions (A- F). Fraction C (800 mg) was subjected to CC over silica gel (70 g, column: 60 x 3 cm) and eluted with hexane followed by hexane/ EtOAc (19:1) to yield four fractions C_a, C_b (153 mg), Cc (200 mg) and C_d. Compound 2 (20.3 mg) was obtained from fraction C_b, 5 (50.7 mg) from fraction C_c and 3 (75 mg) was obtained from C_c by CC [silica gel (30 g), column (50×2 cm), hexane/EtOAc (19:1). Fraction A (9.8 g) was subjected to column chromatography (CC) over silica gel (300 g, column: 80×4 cm) using hexane followed by hexane/EtOAc (39:1) to yield 1(400 mg). Compound 6 (55 mg) and 7 (20 mg) were purified from fraction D by CC silica gel (50 g), column (50×2 cm) eluted with hexane/EtOAc (9:1). Compound 1 (75 mg) and 5 (30 mg) were obtained from fraction F (2g) after purification over CC using silica gel (50 g), column (50×2 cm) with hexane/EtOAc (17:3).

Compound characterization

Compound 1: White powder, $[\alpha]_D^{20}$ = -25° (C = 0,0041 g/mL ; CHCl3); (film) νmax (OH), 3412, 1711 (C=O carboxylic acid), 1291 and 1242 (C-O ester) ; 1H and 13C NMR data see Table 1.

Compound screening

The anticancer activity of compounds1-3 was evaluated at a single dose of 10 µM against the NCI60 panel of human tumour cell line which is derived from nine cancer cell types: leukaemia, lung, melanoma, colon, nervous system, ovary, renal, prostate and breastcancer according to the NCI protocol [16].

Results and Discussion

The present work report the characterization of five tirucallane triterpenoid acids (1-5) including a new 3α-acetoxy-28-hydroxytirucalla-8,24-dien-21-oic acid (1) and two known pentacyclic triterpenoids, α-amyrin and β-amyrin from the resin of *C. schweinfurthii* [Fig. 1]. Compound 1, isolated as a white solid was consistent with the molecular formula $C_{32}H_{50}O_5$ for the compound. The IR spectrum showed a carboxylic acid stretch at 2600-3600 cm^{-1}, a hydroxyl stretch at 3412 cm^{-1} and a carbonyl stretch at 1711 cm^{-1}. The ^{13}C NMR spectrum in conjunction with the DEPT spectrum (Table 1) showed 32 signals including signals for a carboxylic acid carbon (δ_C 182.8), an acetate carbonyl carbon (δ_C 171.2), four signals in the alkene region (δ_C 134.1, 133.3, 132.4 and 123.8), an oxymethine carbon at (δ_C 73.5), an oxymethylene carbon at (δ_C 65.5), four fully substituted carbons, ten methylene and three methine carbon indicating an acetylated triterpenoid di-alcohol. The ^1H NMR spectrum showed an alkene proton resonance at δ_H 5.08 (m), an oxymethine proton resonance at δ_H 5.06 (m), a pair of doublet resonances at δ_H 3.76 and δ_H 3.46 for an oxymethylene group, acetoxy methyl group proton resonance at δ_H 2.07 (s) and six singlet methyl group resonances at δ_H 1.67 and 1.58 for allylic groups, and δ_H 0.98, 0.90, 0.86 and 0.85. A double bond equivalence of 8 was determined for this compound. From this information compound 1 was determined to be a tetracyclic triterpenoid whose two-methyl groups had been oxidised to an alcohol and a carboxylic acid. The use of HMBC spectrum showed that a methyl group proton resonance at δ_C 0.98 and the pair of doublets at δ_H 3.76 and 3.46 correlated with the oxymethine carbon resonances at δ_C 73.5 and a methine carbon resonance at δ_C 46.9. This carbon resonance at δ_C 46.9 showed a correlation in the HMBC spectra with a methyl group proton resonance at δ_H 0.90 (3H-19). Furthermore, the oxymethine carbon resonance at δ_C 73.5 showed a correlation in the HMBC spectrum with the methyl group proton resonances at δ_H 2.07 (s) for the acetoxy group.

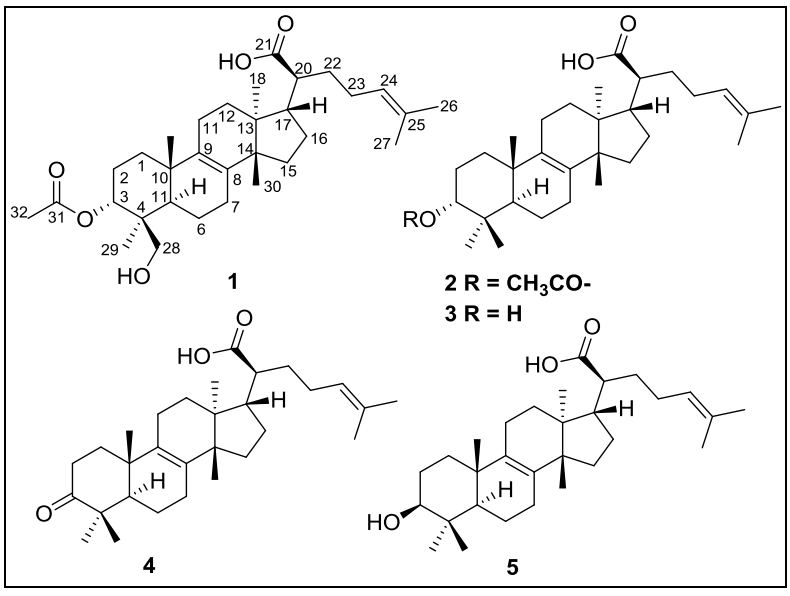

Fig 1: Structures of tirucallane triterpenoid acids isolated from the resin of *Canarium schweinfurthii* Engl.

This information was consistent with an acetoxy group at C-3, hydroxylation at C-28 or C-29 carbon of a triterpenoid skeleton. The C-19 carbon resonance showed a correlation in the HMBC spectrum with a fully substituted alkene carbon resonance at δ_C 134.1, whereas the other alkene carbon resonance at δ_C 133.3 showed a correlation in the HMBC spectrum with another methyl group proton resonance at 0.86, typically at C-30. Furthermore, a methyl group proton resonance at δ_H 0.85 showed correlation in the HMBC spectrum with the 3H-30 methyl group proton resonance. The arrangement of the methyl groups at C-18, C-19, C-29 and C-30 supported a tirucullane triterpenoid as previously observed in the *Canarium* species. On the other hand, two allylic methyl groups at δ_H 1.67 (3H-26) and 1.58 (3H-27) showed correlation in the HMBC spectrum with alkene resonances at δ_C 132.4 and 123.8 for the C-24 and C-25 group of a tirucullane triterpenoid. A carboxylic acid was therefore placed at C-21 position. This compound was therefore determined to possess a 3-acetoxy, a 28-hydroxy later a carboxylic acid at C-21 and di-alkene at C-8 and C-24 position. From the NOESY spectrum the 3H-19 showed a correlation with a doublet methyl group proton resonance at δ_H 3.46. This compound was found to be a new acetoxy derivative of the known compound 2. Compounds 1 and 5 showed weak selective inhibitory effects in the NCI60 human tumour cell line screen at a single dose of 10 μM, with compound 1 the most active. Compound 1 showed 42% growth inhibition against the leukaemia HL-60 (TB) cell line.

The present work report the characterization of five tirucallane triterpenoid acids (1-5) including a new 3α-acetoxy-28-hydroxytirucalla-8,24-dien-21-oic acid (1) and two known pentacyclic triterpenoids, α-amyrin and β-amyrin from the resin of *C. schweinfurthii*. The results from this study confirm the ability of *Canarium* resin to constitute tirucallane triterpenoid acids and that the chemical analysis of the resin of *Canarium* species can be used in the reclassification of the members of the genus.

Table 1: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of 3α-acetoxy-28-hydroxytirucalla-8,24-dien-21-oic acid

No	^{1}H (δ in ppm, J in MHz)	^{13}C (δ in ppm)
1a	1.50 (m)	30.7
1b	1.41 (m)	
2a	1.90 (m)	21.8
2b	1.87 (m)	
3	5.06 (m)	73.5
4	-	42.5
5	1.67 (m, overlap)	46.9
6a	1.68 (m, overlap)	19.1
6b	1.31 (m)	
7a	1.94 (m, overlap)	27.6
7b	2.05 (m)	
8	-	133.3
9	-	134.1
10	-	37.2
11a	1.94 (m)	27.1
11b	1.54 (m)	
12a	1.67 (m)	29.0
12b	1.37 (m)	
13	-	44.1
14	-	49.4
15a	1.55 (m)	29.5
15b	1.23 (m)	
16a	1.81 (m)	23.5
16b	1.69 (m)	
17	2.08 (m)	47.1
18	0.84 s	16.1
19	0.90 s	20.6
20	2.26 (m)	47.8
21	-	182.8
22	1.52 (m)	32.7
22 23a	1.54 (m) 1.97 (m)	 26.2
23b	1.90 (m)	
24	5.08 (t, 7.8)	123.8
25	-	132.4
26	1.67 (s)	25.9
27	1.58 (m)	17.9
28	0.98 (m)	21.5
29a	3.76 (d, 11.3)	65.5
29b	3.46 (d, 11.3)	
30	0.87 (m)	24.7
31	-	171.2
32	2.07 (s)	21.6

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