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TRITERPENES AND 1-(ω-HYDROXYCERATYL)GLYCEROLS FROM *PENTACLETHRA EETVELDEANA* ROOT BARK

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Key Word Index—*Pentaclethra eetveldeana*; Mimosaceae; root bark; 3β-palmityloxy-13β-hydroxyoleanane; lupeyl stearyl ether; 24-palmityloxyoleanonic acid; 1-(26-hydroxyhexacosanoyl)glycerol; 1-(26-ferulyloxyhexacosanoyl)glycerol.

Abstract—Two new monoglycerides, 1-(26-hydroxyhexacosanoyl)glycerol and 1-(26-ferulyloxyhexacosan)-glycerol, and three new fatty acid conjugates of triterpenes, were isolated from the root bark of *Pentaclethra eetveldeana* together with several previously known compounds.

INTRODUCTION

Pentaclethra eetveldeana De Wild. et Th. Dur is a tree of the tropical African forest whose root bark is used in Zairian traditional medicine for the treatment of haemorrhoids and various diseases such as malaria and epilepsy (Barbady-Byla, unpublished results). An earlier investigation reported the presence of saponins in the seeds [1]. In this paper we deal with the isolation and structural elucidation of constituents in the chloroform extract of *P. eetveldiana* root bark.

A combination of column chromatography, TLC and radial chromatography afforded the known compounds friedelin, epifriedelinol [2], 2-hydroxy-4-methoxy-6-*n*-propylbenzoate (ethyl divaricate) (**1**) previously found only in lichens [3, 4], 1-hydroxy-5-methoxyxanthone [5, 6], 2,6-dimethoxybenzoquinone and 2,6-dimethoxy-1-acetonylquinol (**2**) [7]. The new compounds were 3β-palmityloxy-13β-hydroxyoleanane (**3**), lupeyl stearyl ether (**4**), 24-palmityloxyoleanonic acid (**5**), 1-(26-hydroxyhexacosanoyl)-glycerol (**6a**) and 1-(26-ferulyloxy)hexacosanoyl)-glycerol (**6b**).

RESULTS AND DISCUSSION

The ^{1}H NMR spectrum of **3** (see Experimental) displayed signals of eight tertiary methyl groups and a *dd* at δ 4.47 (J = 10 and 6.5 Hz) characteristic of the axial H-3 of a triterpene under an ester function, in this case that of a long chain fatty acid [methyl triplet at δ 0.87, a two-proton triplet at δ 2.28 (α-methylene) and strong absorption at δ 1.24]. The ^{13}C NMR spectrum (Table 1) also exhibited signals of a saturated triterpene incorporating a fatty acid ester moiety equatorially oriented on C-3 (oxymethine doublet at δ 81) and a tertiary hydroxyl (singlet at δ 73.4), which had to be located at C-5, C-9, C-13 or C-18 of the triterpene nucleus. Application of rules governing substituent effects of hydroxyl groups [8] and comparison of the ^{13}C NMR data with those for closely related compounds [9–13] then indicated that the hydroxyl was located at C-13. The CI mass spectrum displayed the $[M + H]^+$ peak at m/z 683, with peaks at m/z 427 and 409 corresponding to the loss of palmitic acid, thus leading to structure **3**. This is the first report of an oleanan-3,13-diol from a natural source.

^{1}H (see Experimental) and ^{13}C NMR spectrometry (Table 1) showed that **4** was a lupeol derivative with an unbranched alkyl group equatorially attached to the oxygen function at C-3 (axial H-3 at δ 3.18, *dd* = 11 and 6 Hz, oxymethine C-3 at δ 79.4 and other ^{1}H and ^{13}C frequencies indicative of a long chain alkyl group). While the CI mass spectrum did not exhibit a $[M + H]^+$ peak, intense peaks at m/z 409 (43.4%) and 271 (100) demonstrated that **4** was lupeyl stearyl ether.

^{1}H and ^{13}C NMR spectrometry showed that **5** was likewise a triterpene incorporating a long chain fatty acid residue. In this case, however, the ester was attached to a methylene carbon (mutually coupled doublets at δ 3.65 and 3.42, J = 11 Hz, and ^{13}C triplet at δ 67.4) with the triterpene unit containing in addition six tertiary methyls, a ketone and a carboxyl (^{13}C singlets at δ 219.8 and 178.6) and a trisubstituted double bond (^{13}C doublet at δ 122.8, singlet at δ 144.4 and ^{1}H triplet at δ 5.30, J *ca* 2 Hz) characteristic of an olean-12-ene. Comparison of the ^{1}H and ^{13}C NMR data with literature data [8, 13] identified the triterpene substructure as that of 24-hydroxyoleanonic acid. The CI mass spectrum displayed a small peak corresponding

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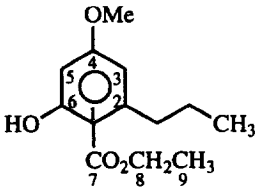

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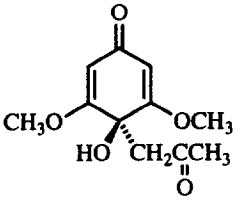

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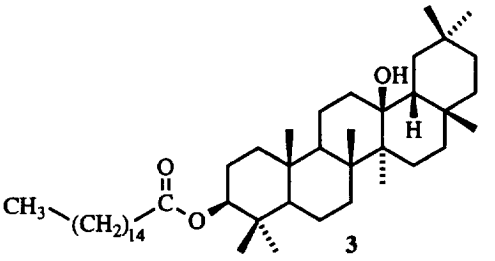

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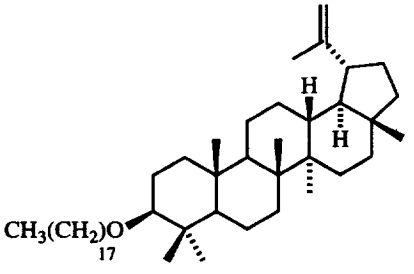

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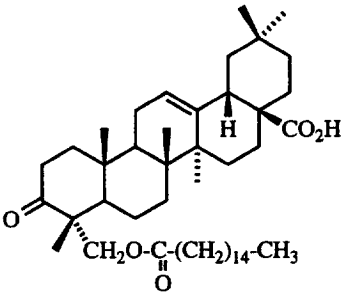

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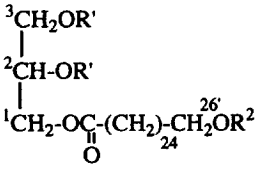

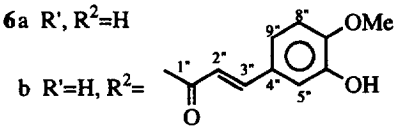

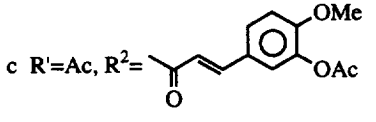

to $[M+H]^+$ at m/z 709 and two intense peaks at m/z 471 and 256, which demonstrated that **5** was 24-palmityloxyoleanonic acid.

Glycerol esters **6a** and **b** are conveniently discussed together. The nature of **6a** was apparent from the ^1H (Table 2) and ^{13}C NMR spectra, which indicated that it was a 1-(ω-hydroxyfattyacyl)glycerol. The PCI-mass spectrum with strong peaks corresponding to $[M]^+$ at m/z 487 for $C_{29}H_{58}O_5$ and $[M-H_2O]^+$ at m/z 469 showed that the esterifying acid was 26-hydroxy-hexacosanoic acid. A mixture of two homologues of **6a** has recently been isolated from *Cinnamum camphorum* [14]. In glyceride **6b** the ω-hydroxyl of **6a**, not the 2- or 3-hydroxyl of the glycerol moiety, was further acylated by ferulic acid as shown by the ^1H spectra of **6b** and its triacetate **6c** (Table 2) and by the ^{13}C NMR spectrum of **6b** (Table 3). The PCI mass spectrum showed that the formula corresponded to $C_{39}H_{66}O_8$, demonstrating that the acid esterifying the glycerol moiety was again 26-hydroxyhexacosanoic acid. A similar glyceride, 1-(22-caffeyloxydocosanoyl)glycerol, has been identified in wax associated with green cotton fibre suberin [15], but not characterized.

EXPERIMENTAL

Plant material. P. eetveldeana was collected near the University of Kinshasa campus. A voucher specimen

Table 1. ^{13}C NMR spectra of compounds **3**, **4** and **5** ($CDCl_3$, 75 MHz)

C	3	4	5	C	3	4	5
1	38.0	38.3	39.0	16	25.1	35.8	23.7
2	23.9	27.6	35.4	17	43.8	43.3	46.8
3	81.0	79.4	219.8	18	50.5	48.6	41.4
4	37.8	39.0	47.1	19	44.9	48.3	46.1
5	55.6	55.7	52.7	20	28.2	151.4	29.4
6	18.4	18.5	19.3	21	32.1	29.8	33.9
7	35.1	34.6	32.3	22	38.7	40.3	32.6
8	41.7	41.1	39.6	23	28.9	28.2	67.4
9	50.3	50.7	49.5	24	16.7	15.5	17.0
10	37.3	37.4	36.8	25	16.3	16.2	15.3
11	21.6	21.1	23.2	26	16.7	16.1	17.2
12	35.8	25.4	122.8	27	15.0	14.7	24.9
13	73.8	37.5	144.4	28	19.4	18.2	178.6
14	48.7	43.1	42.1	29	29.2	109.8	33.2
15	40.5	27.7	27.9	30	22.8	19.5	23.7
1′	173.2	63.4	173.2	14′	31.8		31.8
2′	34.8	33.1	34.1	15′	22.8		22.8
3′	25.3		24.9	16′	14.2	32.1	14.2
4–13′	29.3–29.9		29.3–29.9	17′	—	22.8	—
3–16′		29.5–29.9		18′	—	14.2	—

(C. Evrard No. 6653) is deposited in the herbarium of INERA, Department of Biology, University of Kinshasa, Zaire.

Extraction and isolation. Dried and powdered root bark (1 kg) was successively extracted with *n*-hexane, $CHCl_3$ and MeOH (4 days each). Removal of solvents gave 8 g hexane extract, 8 g $CHCl_3$ extract and 196.7 g MeOH extract. CC of the $CHCl_3$ extract (silica gel, 320 g, eluent: petrol–EtOAc gradient, 50-ml frs) proceeded as follows: Frs 1–10 (petrol), 11–28 (petrol–EtOAc, 9:1), 29–52 (petrol–EtOAc, 4:1), 53–65 (petrol–EtOAc, 5:2), 66–74 (petrol–EtOAc, 1:1) and 75–117 (EtOAc). Frs were monitored by TLC. Those containing identical material were pooled as follows: Frs 28–39 (A), 40–49 (B), 50–66 (C), 67–73 (D), 74–79 (E), 83–85 (G), 86–90 (H) and 91–117 (I). Fr. A (240 mg) was subjected to radial chromatography (eluent: hexane–EtOAc, 6:1, 5-ml frs). Subfrs 10–37 (120 mg) containing identical material were purified by prep TLC (eluent: cyclohexane–$CHCl_3$, 5:1) to afford friedelin (50 mg), **1** (30 mg), 3-epifriedelinol (4 mg), **4** (5 mg) and 1-hydroxy-5-methoxyxanthone (2 mg). Fr. B (55 mg) was processed to give an additional 2 mg of the xanthone and 20 mg **4**. Fr. G (200 mg) was subjected to CC (silica gel, eluent: hexane–EtOAc gradient, 5-ml frs). Subfrs 63–70 after purification by TLC gave 16 mg **5**; similarly subfrs 91–109 and 203–600 afforded, respectively, 2,6-dimethoxyquinone (10 mg) and **2** (20 mg). Similarly, CC of Fr. H followed by TLC gave, from subfrs 83–147, 8 mg **6b**,

Table 2. ^1H NMR spectra of compounds **6a–c** ($CHCl_3$, 500 MHz)

H	6a	6b	6c
1a	4.30 *dd*	4.21 *dd* (12, 5)	4.21 *dd*
1b	4.29 *dd*	4.15 *dd* (12, 5)	4.15 *dd*
2	5.25 *tt*	3.94 *tt* (6.5, 5)	3.92 *tt*
3a	4.16 *dd*	3.70 *dd* (12, 6.5)	3.69 *dd*
3b	4.15 *dd*	3.60 *dd* (12, 6.5)	3.59 *dd*
2′	3.32 *t*	2.34 *t* (7.5)	2.35 *t*
3′	1.61 *m*	1.63 *m*	1.63 *m*
4′–24′	1.25 *br*	1.25 *br*	1.25 *br*
25′	1.69 *quint*	1.6 *m*	1.69 *quint* (7)
26′	4.20 *t* (7)	3.63 *t* (6.5)	4.19 *t* (7)
2″		6.28 *d* (16)	6.38 *d*
3″		7.60 *d* (16)	7.63 *d*
5″		7.03 *d* (2)	7.11 *d*
8″		6.91 *d* (8)	7.05 *d*
9″		7.07 *dd* (8, 2)	7.12 *dd*
OMe*		3.93 *s*	3.86 *s*
OAc*			2.08 *s*, 2.09 *s*
			2.32 *s*

*Intensity of three protons.

Table 3. ^{13}C NMR spectra of compounds **1**, **6a** and **6b**

C	1	C	6a	6b
1	120.3	1	65.6	65.6
2	163.9	2	70.7	70.7
3	99.4	3	63.7	63.7
4	166.3	1′	174.9	174.9
5	112.2	2′	34.4	34.5
6	148.4	3′	25.9	26.2
7	171.5	4′–23′	29.3–29.8	29.3–29.9
8	61.5	24′	25.1	25.1
9	14.2	25′	33.0	32.1
1′	39.2	26′	63.4	64.9
2′	25.2	1″		168.0
3′	14.1	2″		115.3
OMe	55.3	3″		145.2
		4″		127.7
		5″		110.0
		6″		148.6
		7″		147.5
		8″		116.4
		9″		122.5
		OMe		56.3

from subfrs 75–135, 6 mg **6b**, and from subfrs 182–277, 5 mg **6a**. Frs C, D, E and F were not further investigated. Known compounds were identified by MS, ^1H and ^{13}C NMR spectrometry. The previously unreported ^{13}C NMR spectrum of **1** is included in Table 3.

3β-Palmityloxy-13β-hydroxyoleanane (**3**). Gum; MS PCI (isobutane) *m/z* (rel. int.): 683 (M $[C_{46}H_{82}O_3]$ + H^+, 1.5), 665 (7.9), 427 (7.1), 409 (100). ^1H NMR (500 MHz, $CDCl_3$): δ 4.47 (*dd*, $J = 10$, 6.5 Hz, H-3), 2.28 (*t*, $J = 7$ Hz, 2H, H-2′a,b), 1.25 (*br s*, $-CH_{2-n}$), 0.87 (*t*, $J = 6.5$ Hz, 3H, H-16′), 1.21, 1.11, 1.05, 0.94, 0.85, 0.83, 0.83, 0.80 (each *s* and 3H, H-23, 24, 25, 26, 27, 28, 29, 30); ^{13}C NMR spectrum in Table 1.

Lupeyl stearyl ether (**4**). Gum; $[\alpha]_{Hg}$ +17 (*c* 0.21, $CHCl_3$); MS PCI (isobutane) *m/z* (rel. int.): 419 (M $[C_{18}H_{38}O] + H^+$, 43.4), 271 ($C_{18}H_{38}O + H^+$, 100), 253 (54.1). ^1H NMR (500 MHz, $CDCl_3$): δ 4.68, 4.56 (each *d*, $J = 2$ Hz, H-29a,b), 3.63 (*t*, $J = 6.5$ Hz, 2H, H-1′a,b), 3.18 (*dd*, $J = 11$, 5.7 Hz, H-3), 2.36 (*m*, H-19), 1.67 (*br s*, H-30), 1.25 (*br s*, $-(CH_2)_{n-}$), 1.02, 0.96, 0.94, 0.82, 0.78, 0.75 (each *s* and 3H, H-23, 24, 25, 26, 27, 28).

24-Palmityloxyoleanonic acid (**5**). Gum; $[\alpha]_{Hg}$ −11° (*c* 0.09, $CHCl_3$), MS PCI (isobutane) *m/z* (rel. int.): 709 (M $[C_{46}H_{76}O_5] + H^+$, 0.5), 471 ($M + H^+ - C_{16}H_{30}O$, 100), 453 (17), 441 (37), 256 (100). ^1H NMR ($CDCl_3$, 500 MHz): δ 5.30 (*t*, *ca* 2.5 Hz, H-12), 3.65, 3.43 (each *d*, $J = 11$ Hz, H-24a,b), 2.34 (*t*, $J =$ 7.5 Hz, 2H, H-2′a,b), 1.25 (*br*, $-(CH_2)_{-n}$), 0.87 (*t*, $J =$ 7.5 Hz, 3H, H-16′), 1.14, 1.14, 1.01, 0.92, 0.90, 0.82 (each *s*, 3H, H-23, 25, 26, 27, 29, 30).

1-(26-Hydroxyhexacosanoyl)-glycerol (**6a**). Gum; MS PCI (isobutane) *m/z* (rel. int.): 487 (M $[C_{39}H_{66}O_8] + H^+$, 30.6), 469 (89.8); 459 (53.7), 442 (100). ^1H NMR: Table 2; ^{13}C NMR: Table 3.

1-(26-ferulyloxyhexacosanoyl)-glycerol (**6b**). Mp 72–75°; MS CI (isobutane) *m/z* (rel. int.): 663 (M $[C_{39}H_{66}O_8] + H^+$, 41.4), 645 (73.8), 635 (60.9), 631 (16.1), 617 (100). ^1H NMR: Table 2; ^{13}C NMR: Table 3. Triacetate **6c** was prepd in the usual way using Ac_2O–pyridine; ^1H NMR: Table 2; ^{13}C NMR: Table 3.

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