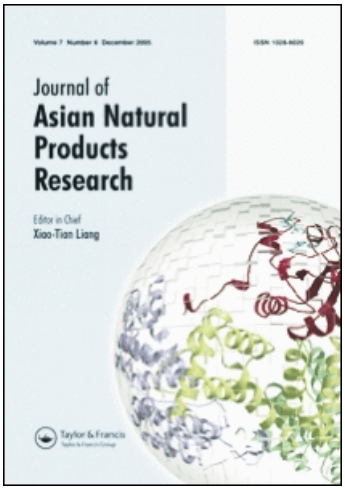

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New phthalates from *Phyllanthus muellerianus* (Euphorbiaceae)

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Species of the genus *Phyllanthus* are known for their medicinal values and many are explored phytochemically. Some of them produce phthalates which usually have antimicrobial properties. This paper deals with the phytochemical investigation on *Phyllanthus muellerianus.* As a result, five compounds, bis(2-ethyloctyl)phthalate (**1**), bis(2-ethylicosyl)phthalate (**2**), 3-friedelanone (**3**), β-sitosterol (**4**), and methyl gallate (**5**), have been isolated and characterized. Metabolites **1** and **2** are new compounds, while **3**–**5** have been isolated for the first time from this source. Structures of all the isolates were established on the basis of MS, 1D and 2D NMR spectral data and in comparison with the reported data.

Keywords: *Phyllanthus muellerianus*; phthalates; triterpenoids; steroids; gallate

1. Introduction

Phyllanthus muellerianus, belonging to the genus *Phyllanthus* of the family Euphorbiaceae, is widely grown in tropical and subtropical regions of the world including China, the Philippines, Cuba, Nigeria, and Guam. Species of the genus *Phyllanthus* have long been used in folk medicine to treat kidney and urinary bladder disorders, intestinal infections, diabetes, and hepatitis B in several parts of the world [1]. They are also known to have anti-inflammatory and antipyretic properties [2]. The roots, leaves, and bark of some *Phyllanthus* species are used to treat diarrhea or dysentery, wart, conjunctivitis, and bronchitis. Although many species of the genus *Phyllanthus* have been explored for their chemical constituents and afford vitamins, mucic acid, tannins [3,4], β-sitosterol and flavanone glycosides [5,6], phenolic glycosides [7], sesquiterpenoids [8], norsesquiterpenoids [9], triterpenoids [10], phenolic acids [11], flavonol, glycosides [12], lignans [13], and phthalates [14], no phytochemical studies have yet been reported on *P. muellerianus*. In this paper, we report the isolation and characterization of new and known constituents from *P. muellerianus*.

2. Results and discussion

The methanolic extract of the shade-dried plant material (6.5 kg) of *P. muellerianus* was evaporated *in vacuo*, suspended in H_2O, and partitioned with hexane, ethyl acetate, and *n*-butanol. The ethyl acetate fraction showed many bands on TLC and

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was subjected to a series of column chromatography to obtain pure compounds **1**–**5** (Figure 1).

Compound **1** was isolated as a yellowish oil whose EI-MS showed molecular ion at *m/z* 446 with other fragments at *m/z* 431, 361, and 289. The molecular formula $C_{28}H_{46}O_4$ was established through the data of HR-EI-MS. The aromatic region of the ^{1}H NMR spectrum of **1** displayed two signals at δ 7.68 (dd, $J = 5.8$, 2.1 Hz) and 7.50 (dd, $J = 5.8$, 2.1 Hz) and oxygenated methylene at δ 4.20 (m), giving indication for the phthalate nature of the molecule [14,15]. The spectrum further showed a methine signal at δ 1.66 (m), two methylenes at δ 1.50 (m) and 1.42 (q, $J = 7.2$ Hz), and two methyls at δ 0.91 (t, $J = 7.3$ Hz) and 0.84 (t, $J = 6.6$ Hz). The same spectrum also showed multiplets about δ 1.22–1.37 for several methylenes in the molecule. The ^{13}C NMR spectrum displayed 13 carbon signals with double intensity (Table 1), which was half of the number observed in the MS spectrum, indicating two identical parts in **1**. The multiplicities of carbons were determined by DEPT experiment and the complete assignments were made on the basis of COSY-45, HMQC, and HMBC techniques (Table 1), and the structure was established as bis(2-ethyloctyl)phthalate (**1**), which is a new compound. Analogs of phthalates are reported as natural products from *Phyllanthus* species [14].

Compound **2** was also obtained as a light yellow oil. The ^{1}H NMR spectral data of **2** was superimposable to those of **1** with the only difference of higher integrals in the region δ 1.29–1.37, while the major difference was observed in the MS spectrum as the HR-EI-MS afforded molecular ion at *m/z* 782.7148 corresponding to the formula $C_{52}H_{94}O_4$. These data indicated that the aliphatic chains in **2** have 12 additional methylenes when compared with **1**. Thus, compound **2** was characterized as bis(2-ethylicosyl)phthalate, which is also a new isolate from this source.

Although phthalates are known as parts of plasticizers and/or bacterial metabolites with antimicrobial properties [15], the members of the genus *Phyllanthus* are also reported to produce these compounds as their secondary metabolites [14].

The known constituents 3-friedelanone (**3**) [16,17], β-sitosterol (**4**), and methyl gallate (**5**) [13] were identified either in comparison with the literature values of NMR and MS data or due to similar TLC profiles with the authentic samples. To the best of our knowledge, these compounds have been reported for the first time from *P. muellerianus*.

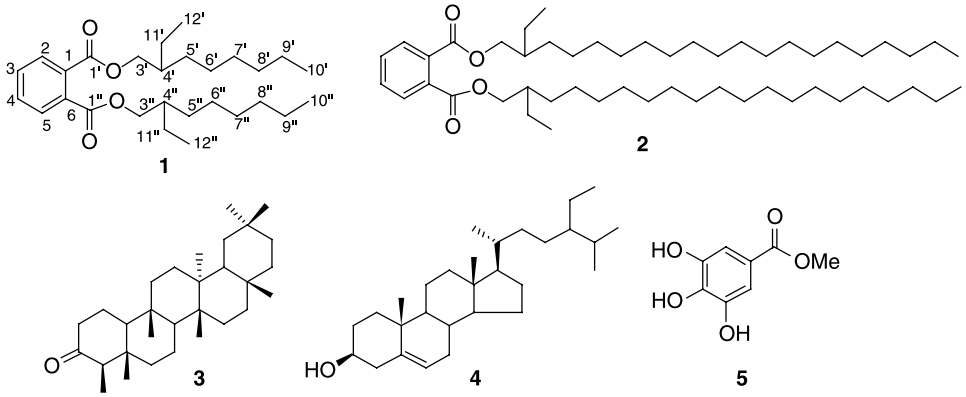

Figure 1. Structures of compounds **1**–**5**.

Table 1. ^{1}H NMR (500 MHz $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) spectral data for **1**.

Position	δ_H	δ_C	Long-range correlations	
			^2J	^3J
1, 6	–	132.5	–	–
2, 5	7.68 (dd, $J = 5.8, 2.1$ Hz)	128.8	C-1, 6, C-3, 4	C-1′, 1″
3, 4	7.50 (dd, $J = 5.8, 2.1$ Hz)	130.8	C-2, 5	C-1, 6
1′, 1″	–	168.1	–	–
3′, 3″	4.20 (m)	68.1	–	C-1′, 1″
4′, 4″	1.66 (m)	38.7	C-3′, 3″, C-11′, 11″	C-12′, 12″
5′, 5″	1.42 (m)	23.7	–	–
6′, 6″, 7′, 7″,8′, 8″	1.22–1.37 (m)	29.6, 22.9	–	–
9′, 9″	1.50 (m)	28.9	–	–
10′, 10″	0.84 (t, $J = 6.6$ Hz)	14.0	–	–
11′, 11″	1.42 (q, $J = 7.2$ Hz)	30.4	C-4′, 4″	C-12′, 12″
12′, 12″	0.91 (t, $J = 7.3$ Hz)	10.9	C-11′, 11″	C-4′, 4″

3. Experimental

3.1 General experimental procedures

The UV spectra were recorded in ethanol on a Hitachi UV-3200 spectrometer (λ_{max} in nm). The IR spectra were recorded on a Shimadzu IR-460 spectrophotometer (ν in cm^{-1}). EI-MS and HR-EI-MS spectra were recorded on a Jeol JMS-HX 110 spectrometer with data system. The ^{1}H NMR spectra were recorded on Bruker AMX-500 instruments using TMS as an internal reference. The chemical shifts are reported in ppm (δ) while the coupling constants *(J)* in Hertz. The ^{13}C NMR spectra were recorded at 125 MHz on the same instrument.

Column chromatography was carried out using silica gel (70–230 and 230–400 mesh; E-Merck, Darmstadt, Germany). Aluminum sheets precoated with silica gel 60 F_{254} (0.2 mm thick; E-Merck) were used for TLC to check the purity of the compounds and were visualized under UV light (254 and 366 nm) followed by ceric sulfate as the spray reagent.

3.2 Plant material

The plant material (whole parts) was collected from the campus of the University of Ibadan, Ibadan, Nigeria and authenticated by Mr Felix Usang of the Forest Research Institute, Ibadan, where a voucher specimen has been deposited under file number FHI106463.

3.3 Extraction and isolation

The shade-dried plant material was ground to coarse powder (6.5 kg) and was soaked in methanol for a period of 8 days twice. The combined extract was suspended in H_2O and was extracted with *n*-hexane, ethyl acetate, and *n*-butanol. The ethyl acetate fraction was subjected to silica gel column chromatography using *n*-hexane, *n*-hexane–chloroform, chloroform, and chloroform–methanol as the mobile phase to obtain 11 fractions (pm-1–pm-11).

Fraction pm-7 eluted from the first column with 50% chloroform in hexane was further subjected to repeated silica gel column chromatography using the same mobile phase to obtain **1** (15 mg) and **2** (10 mg) as yellowish oil.

Main fraction pm-4 obtained from the first column (20% chloroform in hexane) showed one spot on TLC with some impurities and thus was further purified by repeated silica gel column chromatography to obtain **3** (12 mg). Fraction pm-8

eluted with 60% chloroform in hexane was washed with methanol to obtain **4** (50 mg) as a white powder. Fraction pm-9 eluted with pure chloroform from the main column yielded **5** (12 mg) by repeated silica gel column chromatography.

3.3.1 *Bis(2-ethyloctyl)phthalate (**1**)*

UV (Ethanol) λ_{max}, nm (log ε): 226.5 (3.84), 274 (3.09); IR (neat film): 3030, 2810 (C—H), 1732 (CO), 1600–1580 (aromatic C=C), 1450, 1405, 950 cm^{-1}. NMR spectral data, see Table 1. EI-MS: *m/z* 446, 431, 361, 289; HR-EI-MS: *m/z* 446.3320 $[M]^+$ (calcd for $C_{28}H_{46}O_4$, 446.33961).

3.3.2 *Bis(2-ethylicosyl)phthalate (**2**)*

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.65 (2H, dd, $J = 5.6$, 2.2 Hz, H-2, 5), 7.48 (2H, dd, $J = 5.6$, 2.2 Hz, H-3, 4), 4.12 (4H, m, H-3′, 3″), 1.62 (2H, m, H-4′, 4″), 1.42 (4H, m, H-23′, 23″), 1.31–1.25 (remaining CH_2), 0.84 (6H, t, $J = 6.7$ Hz, H-22′, 22″), 0.93 (6H, t, $J = 7.3$ Hz, H-24′, 24″); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 168.3 (C-1′, 1″), 132.5 (C-1, 6), 128.7 (C-2, 5), 130.9 (C-3, 4), 68.1 (C-3′, 3″), 38.6 (4′, 4″), 30.4 (C-23′, 23″), 28.8 (C-21′, 21″), 22.9–22.7 (remaining CH_2), 13.9 (C-22′, C″), 10.9 (C-24′, 24″); HR-EI-MS: *m/z* 782.7148 (calcd for $C_{52}H_{94}O_4$, 782.7152).

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