Terephthalic Acid Bis-(2-ethylhexyl) Ester; an Isomeric Phthalate from *Deinbollia pinnata* Leaves

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The family Sapindaceae are tropical and sub-tropical continental plants. In this study, terephthalic acid bis-(2-ethylhexyl) ester (**1**) and phthalic acid bis-(2-ethylhexyl) ester (**2**) were isolated as isomeric compounds from the ethanolic leaf extracts of *Deinbollia pinnata* for the first time together with methyl gallate **(3)**, ethyl gallate **(4)**, and pyrogallol **(5)**. The separation of the isomeric mixture was by freezing the sub-fraction with methanol for seventy-two hours; followed by filtration, isolation, and purification.

Key words: *Deinbollia pinnata*; isomeric phthalates; phenolics

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Plants are known for their reliance as sources of food, shelter, human health/wellbeing, and income inspiration. The extracts serve as alternative health care resources for more than 80% of the world population [1]. Herbal medicine is often used side by side with modern medicine, with herbal medicine taking the upper hand when the cost of modern medicine is beyond reach [2]. A great attention has been given on the family Sapindaceae, especially *D. pinnata* because of its often use as medication by many traditional practitioners for various diseases in Africa. Roots and leaves of *D. pinnata* are used in folkloric medicine for febrifuge, analgesic, bronchitis intercostal muscle pain, intestinal pain, jaundice, cough, asthma, and infections [3]. Leaf extracts are used in fetus positioning during child birth [4]. However, *D. pinnata* has been neglected for the discovery of its active phytochemicals despite large consumptions of the plant parts, especially the leaves and roots. The elucidation of an isomeric phthalate, spectro-scopically, coincided with the present search for virgin plant phytochemicals' identification, effective microbial degradation, and bioremediation [5-6].

MATERIALS AND METHODS

Plant Material

D. pinnata (Poir.) Schumach. & Thonn leaves were collected from Okehi Local Government Area of Kogi State, Nigeria. The plant part was identified and confirmed at the Biological Department, Federal College of Education Okene Kogi State by Mrs. Aniama S.O.A., a botanist. The plant material was authenticated at Forestry Research Institute of Nigeria Ibadan through comparison with the voucher specimen under the accession number FHI 3251 by Mr. Michael. The leaves were collected, washed, and air dried at room temperature for one month.

Chemicals and Reagents

Solvents used were of general-purpose grade and the reagents used were of analytical grade. The solvents used were *n*-hexane (HEX), dichloromethane (DCM), chloroform ($CHCl_3$), ethyl acetate (EtOAc), acetone (ACE), methanol (MeOH), deuterated acetone (D6), and deuterated chloroform ($CDCl_3$).

EXPERIMENTAL

Extraction of powdered leaves, root bark, and heartwood of *D. pinnata* (Poir.) Schumach. & Thonn with *n*-hexane, ethyl acetate, and ethanol was performed in a sonicator using an ultrasonic-assisted extraction method at intervals of ten-minute agitations using expert design software best conditions [7] and filtered into a bottle and allowed for 24 hrs. Then, filtered using Whatman No. 1 and again filtered and concentrated *in vacuo* at 40°C using a rotary evaporator to dryness. The yields are shown in Table 1. Fractionation and purification of methanol crude extracts were carried out using vacuum liquid chromatography (VLC), column chromatography (CC), and guided by thin layer chromatography (TLC).

Separation of constituents with gravity column chromatography (CC) was carried out on Merck silica gel 60 (70-230 mesh size) for VLC, Merck silica 60 (230-400 mesh size) for CC, and 0.20 mm precoated

gel aluminium plate (DC Kieselgel 60 F254) for TLC. TLC plates were sprayed with vanillin sulphuric acid reagent. The ^{1}H-NMR (400 MHz) and ^{13}C-NMR (400 MHz) spectral data were recorded on Bruker Avance AMX (400 MHz) instrument. TLC spots were visualised with UVITEC Cambridge CB4 IQB. Infrared (IR) spectra were studied using Neat on Perkin-Elmer series 1600 FT-IR spectrophotometer. GC-MS was conducted on Agilent 7820A (G4350) instrument coupled with S9877E. A HP-5MS column with the dimension of 30 m × 0.25 µm × 0.25 µm was set with settings: initial temperature of 100°C, maintained for 10 min; final temperature of 300°C, kept for 10 min; pressure of 10.686 Psi; and septum purge flow of 3.5 mL/min, split ratio 26.8: 0.1, and split flow of 24.228 mL/min. Helium gas was used as a carrier gas. An ionization energy of 70eV was maintained for MS detection. Mass spectral data were obtained from Mass Spectrometry Laboratory, National Institute of Standards and Technology. Melting point was determined using Electrothermal 1A 9100 apparatus.

RESULTS AND DISCUSSION

The crude extracts of *n*-hexane, ethyl acetate, and methanol showed varied physical properties. The agita-sonication extraction yielded 48.25 g of *n*-hexane extract (3.22%), 20.91 g of ethyl acetate extract (1.39%), and 73.92 g of ethanol extract (4.93%) compared to residual yield from Soxhlet method; 1.3 g of *n*-hexane extract (0.09%), 0.7 g of ethyl acetate extract (0.05%), and 2.1 g of ethanol extract (0.14%), as shown in (Table 1). The ethanol extract afforded all isolated compounds. Compound **(1)** was obtained as a light yellow oil; R_f 0.50 in Hex: EtOAc (2:3) and its molecular formula was determined as $C_{24}H_{38}O_4$. The IR spectrum exhibited absorption bands for both compounds (**1**) and (**2**); carbonyl ester at 1726 cm^{-1} (C=O), 2925 cm^{-1} (C-H), and 1121 cm^{-1} (C-O), and aromatic (C-H) at 3010 cm^{-1}. The ^{1}H-NMR spectrum showed four aromatic protons at δ_H 8.11 (2H, *s*, H-11, H-11' / 2H, *s*, H-12, H-12'); two methyleneoxy protons at δ_H 4.26 (2H, *d*, *J* = 5.6 Hz, H-1'); four methylene protons at δ_H 1.33 (2H, *m*, H-2, H-4'), δ_H 1.33 (2H, *m*, H-5'), δ_H 1.33 (2H, *m*, H-7'), and δ_H 1.30 (2H, *m*, H-3'); and six methyl protons at δ_H 0.99 (3H, *t*, *J* = 7.2 Hz, H-6) and δ_H 0.92 (3H, *t*, H-8'). The ^{13}C-NMR and DEPT spectra of (**1**) revealed signals of 12 symmetrical carbons; two carbonyl carbons (δ_C 165.9), two non-protonated carbons (δ_C 134.2), and four accumulated methine (δ_C 129.7), as showed in Table 2. The ^{1}H-H COSY and HMQC spectra enabled the identification of ^{1}H and ^{13}C signals at various positions of methyl, methylene, and methine. The gas chromatography-mass spectroscopy (GC-MS) revealed *m/z* at 390.5 [M^{+}] ($C_{24}H_{38}O_4$) **(1),** while at *m/z* 390.2 **(2)** confirmed theirs as isomers, as displayed in Figure 1.

Elution of DPLEG1-6 (401.4 mg) yielded a light brown solid (24.0 mg, 0.39%) with m.p. of 198-201°C, R_f 0.80 in acetone: chloroform (3:2). Furthermore, a dark purple colour was visualised under UV light at 254 nm which indicated presence of conjugation. The ^{1}H-NMR spectrum showed the presence of aromatic protons at δ_H 7.11 (2H, *s,* H-2, 6) and oxymethyl protons at δ_H 3.79 (3H, *s*). The FTIR spectrum depleted vibrational and stretching properties were O-H (3294 cm^{-1}), C=C (1612 cm^{-1}), and C=O (1689 cm^{-1}). The GC-MS spectrum clearly showed *m/z* = 184.1 [M]$^{+}$, well with the molecular formula $C_8H_8O_5$. The ^{13}C-NMR spectrum indicated six carbon atoms, including five quaternary carbon atoms (C), two methine atoms (CH), and one methyl (CH_2). The presence of seven types of characteristic carbons consisting of ester carbonyl carbons (δ_C 167.0), aromatic oxygenated carbons at δ_C 145.2 (C-3,5), an aromatic carbon at δ_C 137.9 (C-4), an quaternary carbon at the aromatic ring at δ_C 120.7 (C-1), and a methylene aliphatic ester at δ_C 51.0 (C-8).

Continuous elution of the above fraction yielded a beige solid (40.0 mg, 0.65%) with m.p. of 146-151°C and R_f 0.75 in acetone: chloroform (3:2). The IR spectrum exhibited carbonyl ester stretchings of C=O (1692 cm^{-1}), C=C (1611 cm^{-1}), and O-H (3351 cm^{-1}). The GC-MS spectrum observed a molecular ion at *m/z* = 198.1 [M]$^{+}$, corresponding to the molecular formula $C_9H_{10}O_5$ of compound **(4)**. The ^{1}H-NMR spectrum revealed the presence of a methyl group at δ_H 1.29 (3H, *t, J* = 7.2, 14.4 Hz, H-9), aromatic protons at δ_H 7.13 (2H, *s,* H-2, 6), and ethylene protons at δ_H 4.23 (2H, *m,* H-8). The ^{13}C-NMR spectrum showed a carbonyl carbon peak value at (δ_C 166.6). Also, the presence of methyl (δ_C 13.7), ethylene (δ_C 60.1), and other carbon peak values at δ_C 145.1 (C-3,5), δ_C 137.8 (C-4), and δ_C 108.9 (C-2,6) were observed.

Compound **(5)** was obtained by purification of DPLEG17-20 (201.4 mg) as a gray-dark red solid (35.0 mg, 0.57%) with m.p. of 129-132°C and R_f 0.37 in acetone: chloroform (3:2). The GC-MS spectrum revealed its molecular weight at *m/z* = 126.1 [M]$^{+}$, corresponding to the molecular formula $C_6H_6O_3$. The ^{1}H-NMR spectrum revealed the presence of a distinct singlet peak at δ_H 7.15 for 3H, which correspond to C-4, 5, and C-6. The ^{13}C-NMR spectrum showed two quaternary carbons at downfield shifts (δ_C 144.9, δ_C 138.2) assigned to C-2 and C-1, 3. Other aromatic carbons resonated at δ_C 120.6 and δ_C 109.0. The IR spectrum exhibited absorbance bands of Ar-OH stretching at 3355 cm^{-1} and 1611 cm^{-1} (Ar CH=CH). All isolated compounds were compared with previous literatures [8]–[10] for conformity.

NMR Spectroscopic Data for Isolated Compounds

(3) Light brown solid; methyl gallate (24.0 mg, 0.39%); m.p. 198-201°C, R_f 0.80; IR (Neat) ν_{max} cm^{-1}; 3294.18 (O-H), 1612.49 (C=C) and 1689.98 (C=O); ^{1}H-NMR (acetone d6, 400 MHz): δ_H 7.11 (2H, *s*, H-2, H-6). 3.70 (H-8); ^{13}C-NMR (acetone d6, 100 MHz):

δ_C 166.07 (C-7), 145.14 (C-3,5), 137.89 (C-4), 120.77 (C-1), 120.82 (C-2, C-6) and 51.10 (C-8); GC-MS *m/z*: 184.1 $[M]^+$ ($C_8H_8O_5$).

(4) Beige solid; ethyl gallate (40.0 mg, 0.65%); m.p. 146-151°C, R_f 0.75; IR (Neat) ν_{max} cm^{-1}; 1692.40 (C=O), 1611.15 (C=C), and 3351.25 (O-H); ^{1}H-NMR (acetone d6, 400 MHz): δ_H 1.29 (3H, *t*, *J* = 7.2, 14.4 H_Z, H-9), 4.23 (2H, *m*, H-8), 7.13 (2H, *s*, H-2, 6). ^{13}C-NMR (acetone d6, 100 MHz): δ_C 166.63 (C-7), 145.19 (C-3,5), 137.87 (C-4), 108.98 (C-2, C-6), 60.10 (C-8), 13.74 (C-9); GC-MS *m/z*: 198.1 $[M]^+$ ($C_9H_{10}O_5$).

(5) Gray-dark red needle; pyrogallol (35.0 mg, 0.57%); m.p. 129-132°C, R_f 0.37; IR (Neat) ν_{max} cm^{-1}; 3355.08 (Ar-OH), 1611.09 (Ar CH=CH); ^{1}H-NMR (acetone d6, 400 MHz): δ_H 7.15 (3H, *s*, 6-H, 5-H, 4-H). ^{13}C-NMR (acetone d6, 100 MHz): δ_C 144.97 (C-1, C-3), 120.61 (C-5), 138.23 (C-2), 109.06 (C-4, 6); GC-MS *m/z*:126.1 $[M]^+$ ($C_6H_6O_3$).

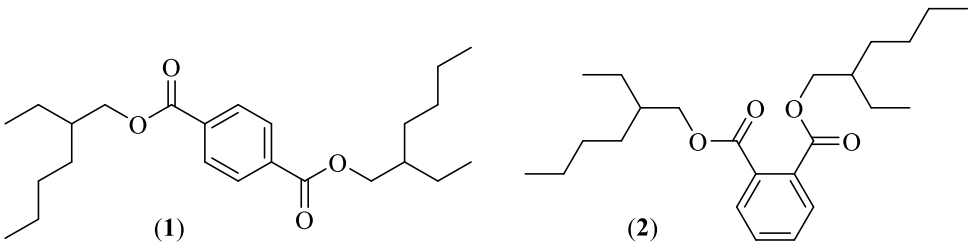
Table 1. Percentage yield of crude extracts of *D. pinnata* leaves

	Extraction time	Extraction temperature 0°C	Solvents (g/%)		
Sonication	35 mins	40	Hex	EtOAc	EtOH
Yield (g) (%)			48.25 (3.22)	20.91 (1.39)	73.92 (4.93)
Soxhlet	Extraction time	Extraction temperature 0°C	Hex	EtOAc	EtOH
Yield (g) (%)	8 hours	80	1.30 (0.09)	0.7 (0.05)	2.1 (0.14)

X mL/1.5 kg proportion solvent to sample ratio was use in the experiment. Values are expressed in both grams / percentages compared to residual.

Table 2. ^{1}H and ^{13}C-NMR data for Compound **(1)**

No.	δ_H (mult. *J* in Hz)	δ_C
1, 4	-	134.25
2, 6	8.11 (2H, *s*)	129.47
3, 5	8.11 (2H, *s*)	129.47
7, 8	-	165.9
1'	4.24 (2H, *m*,)	67.78
2'	1.70 (1H, *dd*, *J* = 6.4 Hz and 12.4 Hz)	38.93
3'	1.30 (2H, *m*)	30.58
4'	1.33 (2H, *m*)	22.95
5'	1.33 (2H, *m*)	24.00
6'	0.99 (3H, *t*, *J* = 7.2 Hz)	14.01
7'	1.27 (2H, *m*)	30.88
8'	0.92 (3H, *t*)	14.01



(**1**) (**2**)

Figure 1. Isomeric dicarboxylic acid esters

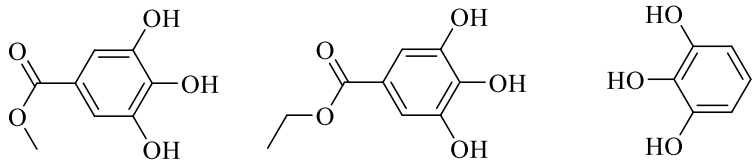


Figure 2. Polyphenolic compounds

CONCLUSION

Plants are known to contained secondary metabolites for survival, growth, development, and protection from a broad spectrum of pathogens. The economic rationale behind extraction, isolation, and structural characterization of phytochemicals has led to the first report of terephthalic acid bis-(2-ethylhexyl) ester (**1**), phthalic acid bis-(2-ethylhexyl) ester (**2**), methyl gallate (**3**), ethyl gallate (**4**), and pyrogallol (**5**) in *Deinbollia pinnata.* The separation of its isomeric mixture has led to the identification of terephthalic acid bis-(2-ethylhexyl) ester. However, based on the massive usage of the plant, a more promising pharmacological profile is needed and the compound concentrates need to be made available to pharmaceutical industries for the development of new medicine.

CONFLICT OF INTEREST

Authors have none to declare

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