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Isolation and Structure Elucidation of Bis(2-Ethylhexyl) Terephthalate from Barks of *Acacia Xanthophloea*

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ABSTRACT

A common and native tree of Eastern and Southern Africa, *Acacia xanthophloea* is a species of tropical plants and a member of the Fabaceae family. Although *A. xanthophloea* is known as a source of tannins, very little is known about its phytochemistry. In this context, the main objective of this work was to isolate and characterize the structures of secondary metabolites found in the bark of this plant. Substantial amounts of bis(2-ethylhexyl) terephthalate [DEHT/ dioctyl terephthalate (DOTP)] were isolated in a fraction of the bark extract while undertaking the isolation of phytochemicals from *A. xanthophloea* barks. The structure of this compound was confirmed by NMR, and mass spectroscopic data. DEHT is a terephthalic acid ester, the main plasticizer that is used to confer elasticity and flexibility to various fiber and plastic products. To the best of our knowledge, this is the first time DEHT has been isolated from the barks of *A. xanthophloea*. This study contributes to the growing evidence of plasticizers in our food and drug sources.

Keywords: Phytochemistry; *Acacia xanthophloea*; Fabaceae; Bis(2-Ethylhexyl) Terephthalate

Abbreviations: KIRDI: Kenya Industrial Research and Development Institute; ACE II PTRE: African Centre of Excellence II in Phytochemicals,Textile and Renewable Energy; JKUAT: Jomo Kenyatta University of Agriculture and Technology; DOTP: Dioctyl Terephthalate; FST: Faculty of Science and Technology

Highlights

- The bark of *Acacia xanthophloea*, a common tree in Eastern and Southern Africa, was found to contain substantial amounts of bis(2-ethylhexyl) terephthalate (DEHT).
- DEHT (bis(2-ethylhexyl) terephthalate), the main plasticizer used in fiber and plastic products is isolated from the barks of this plant.
- This study is the first to report the isolation of DEHT from the bark of *A. xanthophloea* and adds to the growing evidence of plasticizers in our food and drug sources.

Introduction

Acacia xanthophloea, which is also known as *Vachellia xanthophloea*, is a tree in the Fabaceae family and is commonly known in English as the fever tree (local East African names include olerai, kisewa, murera and mwelele). There are roughly 1350 *Acacia* species worldwide, with about 960 of them native to Australia [1]. Africa and the Middle Eastern cosmopolitan nations make up the rest of their distribution [1]. *A. xanthophloea* is native to eastern and southern Africa regions and can be found in Kenya, Botswana, Malawi, Mozambique, Somalia, South Africa, Swaziland, Tanzania, Zambia and Zimbabwe

[2]. Outside of its native region, where the chemistry of most species is still mostly unknown, it has also become a landscape tree in other warm climates. In Kenya, a number of *Acacia* species exist with distribution mainly around Laikipia in Central Kenya and along the Rift valley town of Naivasha. *A. xanthophloea* is a tree that thrives predominantly in the tropical biome with a seasonally dry climate [3]. Various *Acacia* species have historically been utilized for ethnomedical purposes. For instance, *A. nilotica* is used to treat a variety of illnesses, including internal bleeding, diarrhea, and skin conditions [4,5]. *Acacia* species are also widely used as folk medicines in sub-Saharan [6], Chinese [7] and Asian cultures, including Ayurvedic (Indian) and Unani (Greco-Arabic) [8].

There are reports on the use of *Acacia* species (e.g., *A. adsurgens, A. ancistrocarpa, A. bivenosa, A. cuthbertsonii, A. dictyophleba, A. holosericea, A.lysiphloia, A. melanoxylon, A. monticola, A. multisiliqua, Acacia pyrifolia, A. tetragonophylla, A. trachycarpa*, and *A. translucens*) for treating cold, fever, diarrhea, dysentery, and for wound healing. Others such as *A. ixiophylla, A. leptocarpa, Acacia falcata, A. implexa* and *Acacia* inaequilatera have been applied for treating skin diseases, leprosy, rheumatism, stomach disorders, asthma, cancer, and diabetes [9-11] Various *in-vitro* and *in-vivo* pharmacological activities of *Acacia* species such as antiinflammatory, antiviral (including HIV infection), antimicrobial, antioxidant, anti-cancer, antidiabetic, immunomodulatory, hepatoprotective, cardioprotective, and anthelmintic effects have been reported [12]. *Acacia* species are a rich source of bioactive compounds including; phenols, alkaloids, saponins, terpenoids, sterols, polysaccharides, nonprotein amino acids, fatty acids, and miscellaneous organic acids [12]. Various biophenolic subclasses have also been identified in *Acacia*: phenolic acids, flavonoids and tannins [12]. Herein, we report for the first time the isolation of DEHT from the barks of *A. xanthophloea*.

Materials and Methods

General Experimental Procedures

1D and 2D NMR experiments were measured at 500 MHz (^{1}H) and 125 MHz (^{13}C), respectively, on a Bruker spectrometer and referenced to residual solvent peaks. EI-MS spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV) with perfluorkerosine as reference substance for EI-HRMS. Column chromatography (CC) was carried out using silica gel (70–230 mesh, ASTM) as solid phase. Sephadex LH-20 (25–100 µm, Sigma Aldrich) and Preparative thin layer chromatography (PTLC) using silica gel (Merck, 70–230 mesh ASTM) were used for purification steps. TLC was performed on 60 silica gel-coated plates (230–400 mesh, Merck Grade, 20 cm × 20 cm). Compounds on TLC were detected under UV light at 254 or 365 nm and iodine vapor. The barks of *A. xanthophloea* were ground using a Willey mill.

Plant Collection

The stem barks of *Acacia xanthophloea* were collected in February 2021 from Naivasha (0.68350S, 36.40120E), Nakuru County, Kenya, which is 1,884 m (6,181 ft) above sea level and 116 km west of Nairobi. The plant material was identified and authenticated by Mr. Patrick C. Mutiso, a taxonomist from the Faculty of Science and Technology (FST), University of Nairobi herbarium and a voucher specimen (MWCUON2021/001) deposited. The barks were further reduced in size before being air dried in the shade, ground into powder, weighed on a scale, and stored for later use.

Extraction and Isolation

Dried powdered stem bark of *Acacia xanthophloea* (1 kg) was extracted at room temperature by soaking in dichloromethane/methanol (1:1) in a 5 L glass container. The extraction was repeated three times, each extraction taking 24 hours to afford 392 g of the crude extract upon concentration on rotary evaporator. 200 g of the crude extract was fractionated in chromatographic column using silica gel as an adsorbent with gradients of n-hexane-ethyl acetate (10:0, 9.5:0.5, 9:1, 8.5:1.5, 8: 2, 7:3, 6:4, 1:1 and 0:10) and ethyl acetate-methanol (9:1, 8:2 7:3 and 1:1) as eluents to yield 250 fractions (250 mL each). Based on their TLC profiles, the fractions were combined to afford 10 fractions (Fr. 4A-Fr. 4J). Combined fractions Fr. 4E and Fr. 4F (27.8 g), from the column eluting with n-hexane-ethyl acetate (1:1) were passed over Sephadex LH-20 column eluting with dichloromethane and methanol (1:1) and subsequently purified by preparative thin layer chromatography using n-hexane-ethyl acetate (7:3) as the mobile phase, resulting in the isolation of 10.7 mg of bis(2-ethylhexyl) terephthalate (DEHT) (1).

Results and Discussion

The isolated substance was identified as bis(2-ethylhexyl) terephthalate (1) based on the spectroscopic and spectrometric data and comparison with previously published data. Below are the NMR data for the isolated compound (1). Bis(2-ethylhexyl) terephthalate: White amorphous solids; ^{1}H NMR (500 MHz, CD2Cl2): δ_H 8.12 (4H, s, H- 2,3,5 & 6), 4.29 (4H, dd, J = 5.7, 4.4, H-1′/1″), 1.77 (2H, m, H-2′/2″), 1.46 (4H, m, H-3′/3″), 1.30 (4H, m, H-4′/4″), 1.38 (4H, m, H-5′/5″), 1.51(4H, m, H-7′/7″), 0.94 (6H, m, H-6′/6″) and 0.98 (6H, m, H-8′/8″). ^{13}C NMR (125 MHz, C D_2Cl_2) δ_C 166.2 (2 X CO), 134.7 (C-1/4), 129.8 (C-2,3,5 & 6), 68.0 (C-1′/1″), 39.3 (C-2′/2″), 31.0 (3′/3″), 30.1 (4′/4″), 24.4 (7′/7″), 23.4 (5′/5″), 14.2 (6′/6″) and 11.3 (8′/8″); ESIMS m/z 391 [M + H]+; positive ion HRESIMS m/z 391 (calcd for $C_{24}H_{39}O_4$ [M + H]+ , 391) Compound 1 was obtained as white amorphous solids. The molecular formula for the compound was deduced through ES-MS spectrum, which showed a protonated molecular ion $[M + H]^+$ peak at m/z 391 corresponding to the molecular formula $C_{24}H_{39}O_4$ (calcd for $C_{24}H_{39}O_4$, 391). A phthalate was presumed to be present based on the ES-MS diagnostic daughter peaks of 391 at m/z 167 and m/z 149 [13]. The proton ^{1}H NMR spectrum showed a resonance signal in the aromatic region characteristic of a 1,4-disubstituted benzene ring, for the aryl protons H-2/H-5 and H-3/H-6 (δ_H 8.12 s, 4H). Further analysis of the proton-carbon HSQC spectrum showed that these protons were coupled to the corresponding carbon signal at δ_C 129.8 (C-2/C-5

and C-3/C-6). Another resonance attributable to oxygenated methylene protons was also observed further upfield in the ^{1}H NMR spectrum at δ_H 4.29 (4H, dd, J = 5.7, 4.4), which was correlated to a carbon resonance at δ_C 68.0 (C-1′/1″) in the HSQC spectrum. The carbon signal at δ_C 39.3 (C-2′/2″) displayed HSQC connection to the methine protons that were detected at δ_H 1.77 (2H, m, H- 2′/2″).

The COSY spectrum showed a significant correlation between both H-1′/1″ and H-2′/2″, owing to their close proximity, indicating the ester's branched alkyl chains as opposed to their straight chain [13]. Further signals at δ_H 0.94 (6H, s, H-6′/6″), 0.98 (6H, m, H-8′/8″), 1.51 (4H, m, H-7′/7″), 1.38 (4H, m, H-5′/5″), 1.30 (4H, m, H-4′/4″) and 1.46 (4H, m, H-3′/3″) accounted for the protons in the remaining methyl and methylene groups. In addition, key three-bond HMBC correlations clearly linked the aromatic protons and the ox-methylene protons at H-1′/1″ to the ester carbonyl (δ_C 166.2) thus excluding the possibility of the 1- methylheptyl ester isomer. The oxygenated methylene groups were also correlated to methylene carbons at C-2′/2″, C-3′/3″ and C-7′/7″, respectively. Analysis of the ^{13}C NMR spectrum also indicated the presence of 24-carbon signals including ester carbonyl groups (δ_C 166.2, CO) and the C-1/4 quaternary carbons (δ_C 134.7). Thus, based on the spectrometric and spectroscopic data and by comparison with literature values, compound 1 was identified as bis(2-ethylhexyl) terephthalate, previously isolated from Capparis spinosa [14]. However, this is the first time it has been isolated from the Fabaceae family and the genus *Acacia*. In the petrochemical and polymer industrial sectors, bis(2-ethylhexyl) terephthalate (DEHT), a generalpurpose plasticizer and an isomeric compound of di-2-ethylhexyl phthalate (DEHP), is widely used [15]. It belongs to the chemical group of terephthalic acid esters, which are the primary plasticizers employed to give elasticity and flexibility to a variety of fiber and plastic products [16-18]. It has also been used to create a variety of synthetic materials for medical device manufacturing, such as intravenous administration devices [15]. Prior research has identified and isolated DEHT from a number of plant species, including; *Grewia lasiocarpa* [19], *Uncaria rhynchophylla* [20] and *Alnus nitida* [21] as well as the marine fungus *Penicillium griseofulvum* [22]. In addition, other widely used phthalic and terephthalic acid esters that have also been found in many different plants, such as tris(2-ethylhexyl) trimellitate obtained from *Moringa oleifera* [23], diethyl terephthalate derived from *Mangifera indica* [24], and dimethyl terephthalate secluded from *Goniothalamus tapis* [25]. Our research aids in the identification of plasticizers in our food and medication supply chains (Figure 1).

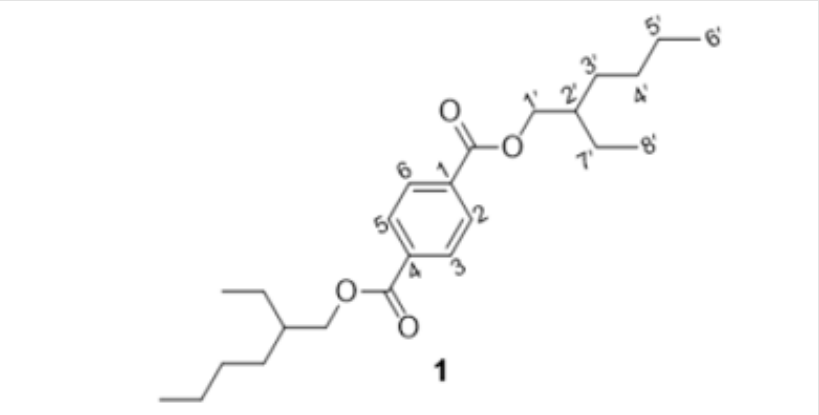

Figure 1.

Conclusion

Phytochemical analysis of a methanol-dichloromethane (1:1) stem bark extract of *A. xanthophloea* led to the identification of one known phenolic phthalate, bis(2-ethylhexyl) terephthalate (1). This is the first time DEHT has been isolated from the Fabaceae family, specifically the genus *Acacia*. DEHT is the main plasticizer employed to provide elasticity and flexibility to a variety of fiber and plastic products. This research contributes to the growing body of evidence showing that plasticizers are present in our food and drug supply.

Declarations

Consent and Ethical Approval

It is not applicable.

Conflict of Interest

Authors have declared that no conflict of interest exist.

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Data Availability Statement

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request.

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