Limonoids from the West African *Trichilia welwitschii* (Meliaceae)

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Abstract

The seeds of *Trichilia welwitschii* C.DC. (Meliaceae) yielded three limonoids, dregeanin DM4 (1), reported here from a natural source for the first time, the known rohituka 3 (2) and trichilia lactone D5 (3). The leaves yielded 28,29-dinorcycloart-24-ene-3,4,6-triol (4), sitosterol-3-*O*-β-D-glucoside, 4-hydroxy-*N*-methyl-*L*-proline, stigmasterol and sitosterol.

Authors Keywords: Meliaceae; *Trichilia welwitschii* C.DC., limonoids; chemotaxonomy

1. Subject and source

Trichilia welwitschii C.DC. is a West African tree growing up to 15m high in the Terra Firma Forests of Nigeria, Cameroon, Angola and Gabon (Louppe *et al.*, 2008). The seeds and leaves of *T. welwitschii* were collected in July 2009 from the Simbock locality in the Central region of the Republic of Cameroon. Plant material was identified by Dr. Nolle, a plant taxonomist at the *Institut de recherches Médicales et d'études des Plantes Médicinales (IMPM)*. A voucher specimen was deposited (31288/SRF Cam) at the National Herbarium of Cameroon.

2. Previous work

There are no previous reports on the phytochemical investigation of *T. welwitschii*. However, *T. heudelottii* Planch. ex Oliv. (syn. *T. monadelpha* (Thonn.) J.J.de Wilde), which is often confused with *T. welwitschii* (Louppe et al., 2008), has been investigated. Taylor has divided limonoids into groups according to which ring of the triterpenoid skeleton has been oxidized (Taylor, 1984). *T. heudelottii* has been reported to yield heudelottin C, E and F (Okorie and Taylor, 1968; Okorie and Taylor, 1972) belonging to the azadirone class of ring A-D intact limonoids, heudebolin (Okorie and Taylor, 1968) of the nimbin class of ring C-oxidized limonoids and dregeanin (Adesida and Okorie, 1973) belonging to the ring A and B oxidized prieurianin class of limonoids.

3. Present study

Air dried and powdered seeds (1.5 kg) and leaves (2.5 kg) of *T. welwitschii* were macerated and extracted separately at room temperature for 24 hours with a mixture of CH₂Cl₂: MeOH (1:1) to yield, upon evaporation of the solvent, 300 g and 250 g respectively of greenish seed and leaf extracts. Flash chromatography over silica gel (230-400 mesh) of 300 g of the seed extract using a gradient of n-hexane, n-hexane-EtOAc (1:1), EtOAc, EtOAc-MeOH (1:1) and MeOH yielded 5 fractions, F₁ (30 g), F₂(100 g), F₃ (50 g), F₄ (60 g) and F₅ (45 g). Fractions F₁ and F₂ were complex mixtures of low polarity compounds which were not examined further, fraction F₃ (50 g) was separated using column chromatography over silica gel using a gradient solvent system of n-hexane and EtOAc to yield stigmasterol (13 mg), sitosterol (25 mg) and dregeanin DM4 (**1**) (Connolly *et al.*, 1979) (115 mg). Fraction F₄ (60 g) was separated in a similar manner to give white needles of rohituka 3 (**2**) (Gunatilaka *et al.*, 1998, Zhang *et al.*, 2007) (50 mg) and a white solid identified as trichilia lactone D5 (**3**) (Connolly *et al.*, 1979; MacLachlan *et al.*, 1982) (60 mg).

The crude leaf extract (250 g) of *T. welwitschii* was fractionated by flash column chromatography over silica gel (230-400 mesh) and eluted successively using n-hexane, hexane-EtOAc (1:1), EtOAc, EtOAc-MeOH (1:1) and MeOH to give five fractions F₆ (25 g), F₇ (70 g), F₈ (85 g), F₉ (30 g) and F₁₀ (40 g) . Fraction F₇ (70 g) was further subjected to column chromatography over silica gel and eluting with n-hexane/EtOAc mixture in increasing polarity to yield 28,29-

dinorcycloart-24-ene-3 β ,4 α ,6 α -triol (**4**) (Brader *et al.*, 1998) (60 mg). Fraction F₈ (85 g) was also separated in a similar manner to give sitosterol-3 β -*O*-glucopyranoside (100 mg) while fraction F₉ (30 g) afforded 4-hydroxy-*N*-methyl-L-proline (Winkler, 2006) (50 mg) unusually isolated from a member of the Meliaceae family. The structures of the isolated compounds were established by analysis of their spectroscopic and spectrometric data and comparison with values reported in the literature as referenced. Dregeanin DM4 was first identified from the alkaline hydrolysis of dregeanin isolated from *T. prieuriana* (Connolly *et al.*, 1979). Its complete ¹H and ¹³C NMR spectral assignments have not been reported in the literature. The ¹H and ¹³C chemical shifts for dregeanin DM4 are as follows: ¹H NMR (500 MHz, CDCl₃): δ 7.32 (1H, s, H-23), 7.09 (1H, s, H-21), 6.10 (1H, s, H-22), 5.55 (1H, d, 9.4 Hz, H-12), 5.42 (1H, s, H-30a), 5.35 (1H, s, H-30b), 4.22 (1H, d, 11.0 Hz, H-11), 4.21 (1H, d, 11.3 Hz, H-29a), 4.11 (1H, dd, 5.4, 11.0 Hz, H-1), 3.97 (1H, d, 11.3 Hz, H-29b), 3.91 (1H, s, H-15), 3.78 (1H, s, H-2'), 3.20 (1H, d, 8.9 Hz, H-9), 3.01 (1H, m, H-2a), 2.96 (1H, m, H-17), 2.81 (1H, m, H-2), 2.80 (1H, m, H-6a), 2.71 (1H, m, H-6b), 2.42 (1H, dd, 9.3, 9.3 Hz, H-5), 2.26 (1H, dd, 7.1, 14.1 Hz, H-16a), 1.84 (1H, dd, 11.0, 14.1 Hz, H-16b), 1.67 (1H, m, H-3'), 1.63 (3H, s, H-28), 1.35 (1H, m, H-4'a), 1.24 (3H, s, H-19), 1.18 (1H, m, H-4'b), 0.92 (3H, d, 6.9 Hz, H-6'), 0.82 (3H, t, 7.5 Hz, H-5'), 0.79 (3H, s, H-18) and ¹³C NMR (125 MHz, CDCl₃): 175.3 (C-1'), 171.3 (C-7), 167.7 (C-3), 142.9 (C-23), 140.3 (C-21), 137.2 (C-8), 122.5 (C-20), 121.2 (C-30), 111.4 (C-22), 79.9 (C-11), 79.8 (C-1), 79.2 (C-4), 75.1 (C-2'), 74.7 (C-12), 72.1 (C-29), 71.5 (C-14), 59.3 (C-15), 55.6 (C-9), 51.1 (C-10), 44.7 (C-5), 44.5 (C-13), 39.5 (C-3'), 38.0 (C-1'), 37.7 (C-2), 33.4 (C-16), 31.2 (C-6), 26.7 (C-28), 24.0 (C-4'), 17.5 (C-19), 15.0 (C-6'), 13.5 (C-5'), 12.0 (C-12).

Compounds **1**, **2**, **3** and **4** were submitted to the NCI NC59 anticancer screen and showed no significant activity.

Figure 1: Compounds isolated from *T. welwitschii*

4. Chemotaxonomic significance

The tropical genus *Trichilia* is one of the 50 genera in the Meliaceae family (Gouvea *et al.*, 2008). *Trichilia* comprises 90 species distributed in tropical America, continental Africa and Madagascar. The Meliaceae family is constantly under review and several rearrangements have been proposed (Harms, 1940, Pennington and Styles, 1975, Muellner *et al.*, 2006). Harms (1940) divided the Meliaceae into three subfamilies: the Cedreloideae subfamily with two tribes, Cedreleae and Ptaeroxyleae; the Swietenioideae subfamily with no tribes and the Melioideae with five tribes, Carapeae, Turraeeae, Vavaeeae, Melieae and Trichilieae (Harms, 1940). In 1975 Pennington and Styles removed Ptaeroxyleae from the family and moved Cedreleae and Carapeae into the Swietenioideae subfamily and elevated Harm's Trichileae to a subfamily and divided it to four tribes, Trichilieae, Aglaieae, Guareeae and Sandoriceae. More recently Muellner and co-workers proposed the addition of Aglaieae, Guareeae and Sandoriceae to Harm's Melioideae subfamily, and renamed Harm's Swietenioideae as the Cedreloideae subfamily with three tribes Cedreleae, Swietenieae and Xylocarpeae (Muellner *et al.*, 2006). The Meliaceae family is chemically distinguished by the occurrence of different classes of limonoids. Muellner's Melioideae and Cedreloideae subfamilies are clearly chemically distinguished. Four classes of highly oxidized ring B fissioned limonoids have been reported from members of the African *Trichilia* genus, the trichilin class from *T. roka* and *T. emetica*, the nimbolinin class from *T. heudelotii*, the vilasinin class from *T. rubescens* and *T. roka* and the prieurianin class from *T. prieuriana*, *T. dregeana*, *T. emetica*, *T. heudelotii* and *T. roka* (Tan and Luo, 2011). The occurrence of limonoids, dregeanin DM4 (**1**), rohituka 3 (**2**) and trichilia lactone D5 (**3**), belonging to the prieurianin class of limonoids from the seeds of *T. welwitschii* is in accordance with the observed trend (Tan and Luo, 2011; Taylor, 1981). *T. welwitschii* is often confused with *T. heudelotii* (syn. *T. monadelpha*), both endemic to west and central African forests (Louppe *et al.*, 2008; Klopper *et al.*, 2006). The isolation of dregeanin DM4 (**1**), rohituka 3 (**2**) and trichilia lactone D5 (**3**) all belonging to prieurianin class from *T. welwitschii* but nimbolinin-type limonoids from *T. heudelotii* shows a clear chemical distinction between the two species.

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