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Triterpene saponins of the root bark of *Olax obtusifolia* De Wild

David Pertuit[a], Anne-Claire Mitaine-Offer[a], Tomofumi Miyamoto[b], Chiaki Tanaka[b], Clément Delaude[c], Marie-Aleth Lacaille-Dubois[a,*]

[a] Laboratoire de Pharmacognosie, PEPITE EA 4267, UFR des Sciences de Santé, Université de Bourgogne Franche-Comté, 7, Bd Jeanne d'Arc, BP 87900, 21079, Dijon Cedex, France
[b] Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, 812-8582, Japan
[c] Centre de Recherche Phytochimique, Université de Liège, Institut de Chimie B-6, Sart Tilman, B-4000, Liège I, Belgium

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ABSTRACT

Four undescribed triterpenoid saponins together with five known and oleanolic acid were isolated from root bark of *Olax obtusifolia* De Wild. Their structures were elucidated by spectroscopic methods including 1D and 2D NMR experiments, in combination with mass spectrometry as 3-*O*-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-β-D-glucuronopyranosyloleanolic acid, 3-*O*-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-β-D-glucuronopyranosyloleanolic acid 28-*O*-β-D-glucopyranosyl ester, 3-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucuronopyranosyloleanolic acid and 3-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucuronopyranosyloleanolic acid 28-*O*-β-D-glucopyranosyl ester.

1. Introduction

The genus *Olax* (Olacaceae) consists of around 50 species found in the tropical regions of Africa and Asia. The Olacaceae family along with Loranthaceae, Misodendraceae, Santalaceae and Opiliaceae belong to the Santalales order (APG III, 2009). Until now, little work has been done towards the isolation of saponins from the genus *Olax*. Olaxoside, a triterpene saponin has been isolated and characterized from the methanol extract of the leaves, roots and bark of *O. andronensis, O. glabliflora* and *O. psittacorum* (Forgacs and Provost, 1981). Other previous studies reported that 5 species from Zaire, *O. subscorpioidea*, *O. wildemanii*, *O. gambecola*, *O. angustifolia* and *O. latifolia* contained in the root bark saponins which after acid hydrolysis gave oleanolic acid, glucose, xylose and rhamnose (Delaude and Huls, 1982). Furthermore, the roots of *Olax dissitiflora* growing in Mozambique yielded saponins which after acid hydrolysis afforded oleanolic acid, hederagenin, 21-epimacherinic acid (Gabetta et al., 1974) and the saponins of *Olax obtusifolia* growing in Democratic Republic of the Congo (DRC) released by acid hydrolysis oleanolic acid, hederagenin and machaerinic acid and glucose, xylose and rhamnose and glucuronic acid as sugars (Delaude and Huls, 1982). As an extension of these works, we undertook a phytochemical study of *O. obtusifolia* aiming to draw some chemotaxonomic conclusions. *O. obtusifolia* De Wild (Olacaceae) is a shrub, or tree, growing in the occidental part of Zambia and High Shaba in the DRC (Delaude and Huls, 1982). Leaves are ovate to elliptic, light green, hairless. Flowers are in small axillary clusters, rarely solitary, creamy-white. The fruit is a spherical drupe, c. 2.5 cm in diameter, yellow-orange when ripe (Palgrave, 2002). A few informations are available on its medicinal properties. However, other species such as *Olax mannii* are used in traditional medicine for the treatment of a variety of ailments and for the ethnomedicinal management of both cancer and inflammation (Okoye et al., 2015, 2016). A decoction of the leaves and roots of *O. mannii* is used for the treatment of fever, yellow fever and snake bite (Burkill, 1997). An extract of *Olax subscorpioidea* was reported to possess a potent analgesic action mediated centrally and peripherally (Adebayo Adeoluwa et al., 2014).

Here, we report the isolation and structure elucidation of four undescribed triterpene saponins together with five known ones and the triterpene oleanolic acid from the root bark of *O. obtusifolia* De Wild. Their structures were elucidated by spectroscopic methods including 600 MHz 1D and 2D NMR experiments (^{1}H, ^{13}C, HSQC, HMBC, COSY, TOCSY, ROESY) in combination with ESI- and HR-ESI-MS and by comparaison of their physical and spectral data with literature values.

2. Results and discussion

The saponin fraction obtained from the 80% aqueous methanolic extract of the root bark of *Olax obtusifolia* was fractionated by repeated

* Corresponding author.
E-mail address: m-a.lacaille-dubois@u-bourgogne.fr (M.-A. Lacaille-Dubois).

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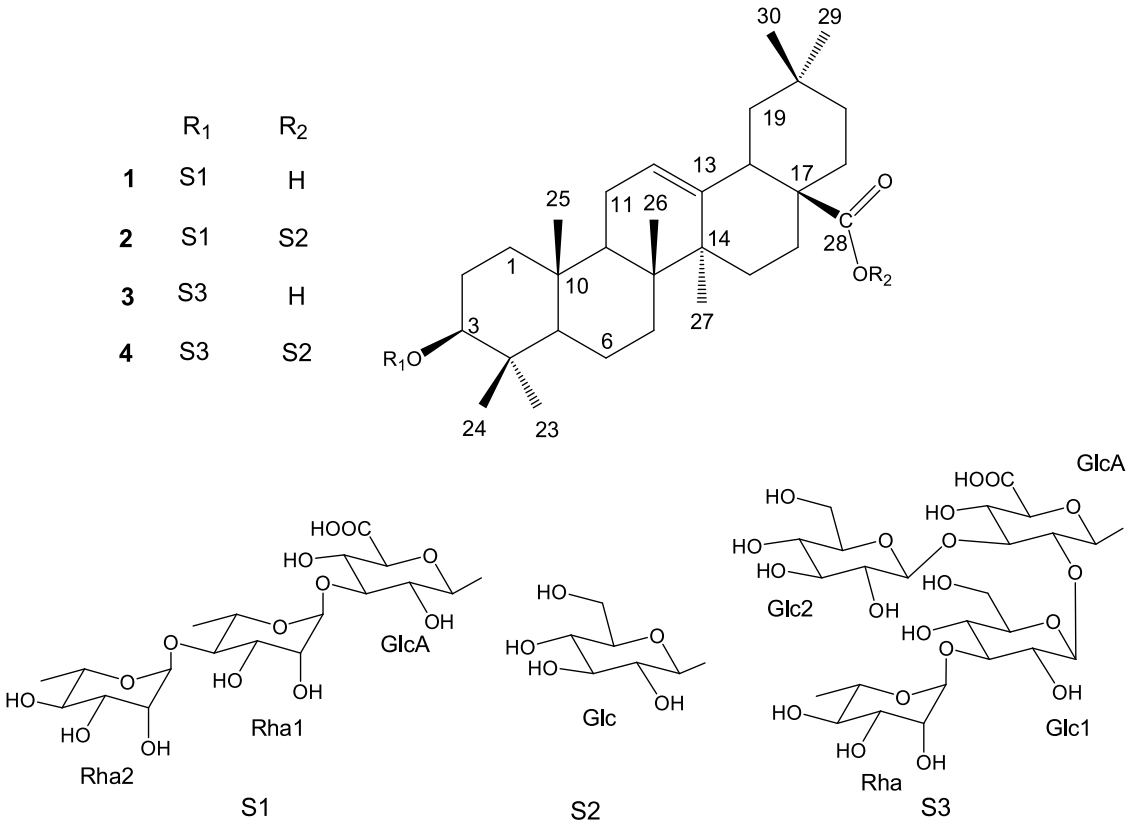

Fig. 1. Saponins from root bark of *Olax obtusifolia*.

medium pressure liquid chromatography (MPLC) on normal and RP-18 silica gel and semi-preparative HPLC using RP-18 silica gel yielding four undescribed compounds **1** – **4** (Fig. 1). Furthermore, oleanolic acid (Mahato and Kundu, 1994), and five known saponins were isolated and identified by comparison of their spectral data with literature values as oleanolic acid-28-*O*-β-D-glucopyranosyl ester (Nie et al., 1984), 3-*O*-β-D-glucuronopyranosyloleanolic acid (Kizu et al., 1985), 3-*O*-β-D-glucuronopyranosyloleanolic acid 28-*O*-β-D-glucopyranosyl ester (Mizui et al., 1990), 3-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-glucuronopyranosyloleanolic acid and 3-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-glucuronopyranosyloleanolic acid 28-*O*-β-D-glucopyranosyl ester (Schteingart and Pomilio, 1984).

Compounds **1** – **4** were isolated as white amorphous powders. The monosaccharides obtained by acid hydrolysis of each compound were identified by comparison on TLC with authentic samples as glucuronic acid and rhamnose for **1**, glucuronic acid, glucose and rhamnose for **2** – **4**. The absolute configurations were determined by GC analysis (Hara et al., 1987) to be D for all the sugars excepted for the rhamnose (L-configuration). The $^{3}J_{H\text{-}1,H\text{-}2}$ values in the ^{1}H NMR spectrum of the glucuronic acid and glucose in their pyranose form (6.2–8.5 Hz) indicated their β anomeric configuration and the large $^{1}J_{H\text{-}1,C\text{-}1}$ value of the rhamnose (168 Hz) confirmed that the anomeric proton was equatorial (α-pyranoid form).

Compound **1** exhibited in the HR-ESI-MS a quasi-molecular ion peak at *m/z* 947.4986 $[M + Na]^+$ (calcd. 947.4980) compatible with the molecular formula $C_{48}H_{76}O_{17}$. Compound **1** showed in ESI-MS spectrum (positive-ion mode) a pseudo-molecular ion peak at *m/z* 947 $[M + Na]^+$ indicating a molecular weight of 924. The ^{1}H and ^{13}C NMR spectra of the aglycon of **1** displayed resonances due to seven angular methyl groups at δ_H 1.03 (s, H_3-23), 0.83 (s, H_3-24), 0.93 (s, H_3-25), 0.81 (s, H_3-26), 1.14 (s, H_3-27), 0.89 (s, H_3-29) and 0.93 (s, H_3-30) showing correlations in the HSQC spectrum with their corresponding carbons at δ_C 27.1 (C-23), 15.6 (C-24), 14.5 (C-25), 16.3 (C-26), 24.9 (C-27), 32.2 (C-29) and 22.6 (C-30) (Table 1). Furthermore, other characteristic signals were observed such as one olefinic proton at δ_H 5.23 (1H, br t, H-12) showing HSQC correlation with δ_C 122.1 (C-12), and one oxygen bearing methine proton signal at δ_H 3.17 (dd, J = 4.6, 11.7 Hz, H-3). Extensive 2D NMR analysis confirmed the structure of the aglycon to be oleanolic acid (Koz et al., 2010; D'Agostino et al., 1993). The ^{1}H NMR spectrum of **1** showed three anomeric proton signals at δ_H 4.33 (d, J = 8.2 Hz), 5.17 (d, J = 1.7 Hz) and 5.18 (br s) giving correlations with their anomeric carbons in the HSQC spectrum at δ_C 105.1, 100.7 and 101.7 respectively. Complete assignments of each sugar unit were achieved by extensive 1D and 2D NMR analyses and GC analysis (see experimental) allowing the characterization of two α-L-rhamnopyranosyl (Rha1 and Rha2) and one β-D- glucuronopyranosyl (GlcA) (Table 2). The HMBC correlations at δ_H/δ_C 4.33 (GlcA H-1)/89.3 (Agly C-3), 5.17 (Rha1 H-1)/81.9 (GlcA C-3) and 5.18 (Rha2 H-1)/ 79.7 (Rha1 C-4) suggested the sequence $Rha2^{1\text{-}4}Rha1^{1\text{-}3}GlcA^{1\text{-}3}$aglycon, confirmed by ROESY correlations at $\delta_H/\ \delta_H$ 4.33 (GlcA H-1)/3.17 (Agly H-3), 5.17 (Rha1 H-1)/ 3.50 (GlcA H-3) and 5.18 (Rha2 H-1)/ 3.50 (Rha1 H-4). ^{13}C NMR values of GlcA C-3 seems to be low, but was in accordance with literature data (Crespin et al., 1993; Borel and Hostettmann, 1987; Schteingart and Pomilio, 1984). Thus, the structure of **1** was elucidated as 3-*O*-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-β-D-glucuronopyranosyloleanolic acid.

Extensive 2D NMR analysis of **2**-**4** allowed the identification of their aglycons as oleanolic acid (Table 1) (Koz et al., 2010).

Compound **2** exhibited in the HR-ESI-MS a quasi-molecular ion peak at *m/z* 1109.5501 $[M + Na]^+$ (calcd. 1109.5508) compatible with the molecular formula $C_{54}H_{86}O_{22}$. Compound **2** showed in ESI-MS spectrum (positive-ion mode) a pseudo-molecular ion peak at *m/z* 1109 $[M + Na]^+$ indicating a molecular weight of 1086. Extensive 2D NMR analysis (Table 1) and GC analysis showed that compounds **1** and **2** differed only by the presence of one additional β-D-glucopyranosyl (Glc) unit in compound **2**. The shielded anomeric carbon signal at δ_C 94.2 (Glc C-1) suggested the Glc residue to be linked at C-28 of the aglycon