Triterpene saponins of the root bark of Olax obtusifolia De Wild

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\begin{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 3-0-\(\alpha\)-l-rhamnopyranosyl(1→4)-\(\alpha\)-l-rhamnopyranosyl(1→3)-\(\beta\)-d-glucuronopyranosyloleanolic acid, 3-0-\(\alpha\)-l-rhamnopyranosyl(1→4)-\(\alpha\)-l-rhamnopyranosyl(1→3)-\(\beta\)\(-\)d-glucuronopyranosyloleanolic acid 28-O\(-\beta\)-d-glucopyranosyl ester, 3-0-\(\alpha\)-l-rhamnopyranosyl(1→3)-\(\beta\)-d-glucopyranosyl(1→2)-[\(\beta\)-d-glucopyranosyl(1→3)]-\(\beta\)-d-glucuronopyranosyloleanolic acid and 3-0-\(\alpha\)-l-rhamnopyranosyl(1→3)-\(\beta\)-d-glucuronopyranosyl(1→2)-[\(\beta\)-d-glucopyranosyl(1→3)]-\(\beta\)-d-glucuronopyranosylolanolic acid 28-O\(-\beta\)-d-glucopyranosyl ester.
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\section{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. Olaxiside, a triterpene saponin has been isolated and characterized from the methanol extract of the leaves, roots and bark of \textit{O. andromensis}, \textit{O. glabiflora} and \textit{O. psittacorum} (Forcags and Provost, 1981). Other previous studies reported that 5 species from Zaire, \textit{O. subscorpioidea}, \textit{O. wildemannii}, \textit{O. gambecola}, \textit{O. angustifolia} and \textit{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 \textit{Olexisstiflora} growing in Mozambique yielded saponins which after acid hydrolysis afforded oleanolic acid, hederaegenin, 21-epimacheric acid (Gabetta et al., 1974) and the saponins of \textit{Olexisstiflora} growing in Democratic Republic of the Congo (DRC) released by acid hydrolysis oleanolic acid, hederaegenin and machaericin 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 \textit{O. obtusifolia} aiming to draw some chemotaxonomic conclusions. \textit{O. obtusifolia} De Wild (Olacaceae) is a shrub, or tree, growing in the ocidential 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 \textit{Olex mani} 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 \textit{O. mani} is used for the treatment of fever, yellow fever and snake bite (Burkill, 1997). An extract of \textit{Olex 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 triterpenoid saponins together with five known ones and the triterpene oleanolic acid from the root bark of \textit{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.

\section{Results and discussion}

The saponin fraction obtained from the 80% aqueous methanolic extract of the root bark of \textit{Olex obtusifolia} was fractionated by repeated

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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-α-L-rhamnopyranosyl-(1→3)-β-D-glucuronopyranosyloleanolic acid (Kizu et al., 1985), 3-O-α-L-rhamnopyranosyl-(1→3)-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 $^1$H NMR spectra of the glucuronic acid and glucose in their pyranose form (6.2–8.5Hz) indicated their β anomeric configuration and the large $^1$JH-1,C-1 value of the rhamnose (168Hz) 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 δH 1.03 (s, H-23), 0.83 (s, H-24), 0.93 (s, H-25), 0.81 (s, H-26), 1.14 (s, H-27), 0.89 (s, H-29) and 0.93 (s, H-30) showing correlations in the HSQC spectrum with their corresponding carbons at δC 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 δH 5.23 (1H, br t, H-12) showing HSQC correlation with δC 122.1 (C-12), and one oxygen bearing methine proton signal at δH 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 δH 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 δC 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 δH/δC 4.33 (GlcA H-1)/89.3 (Agl 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 Rha21-4Rha11-3GlcA1-3aglycon, confirmed by ROESY correlations at δH/δH 4.33 (GlcAH-1)/3.17 (AglH-3), 5.17 (Rha1 H-1)/3.50 (GlcAH-3) and 5.18 (Rha2 H-1)/ 3.50 (Rha1 H-4). $^{13}$C NMR values of GlcAC-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 δC 94.2 (Glc C-1) suggested the Glc residue to be linked at C-28 of the aglycon.