# Excelsoside: A New Benzylic Diglycoside from the Leaves of *Milicia excelsa*

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A new benzylic diglycoside was isolated from the leaves of *Milicia excelsa* and identified as 3,4-dimethoxybenzyl $\beta$-D-xylopyranosyl(1→2)-$\beta$-D-glucopyranoside (**1**). It was obtained together with four known secondary metabolites including lupeol acetate (**2**), ursolic acid (**3**), triacontyl (*E*)-ferulate (**4**), and 2-(3,5-dihydroxyphenyl)benzofuran-5,6-diol (**5**). Their structures were determined based on their spectroscopic data and by comparison with those reported in the literature.

*Key words: Milicia excelsa*, Glycoside, Structure Elucidation, NMR Spectroscopy

## Introduction

Medicinal plants are still being used by sub-Saharan communities as alternative and complementary means to cure diseases. The identification of secondary metabolites from medicinal plants is a rewarding task which allows the scientific evaluation of the empirically observed bioactivity as well as the potential toxicity of active constituents. Although biological effects of plant extracts may not be traced back to a single metabolite, such phytochemical and phytopharmacological investigations have proven very fruitful for the neighbouring chemical disciplines. Our preliminary investigation of the roots of *Milicia excelsa* Welw. C. C. Berg (Moraceae), a large tree of up to 50 m height and 2 m in diameter (Ouinsavi and Sokpon, 2010), led to the isolation and characterization of a new flavonoid and other related compounds (Ouete *et al*., 2013). The presence of these polyphenols in *M. excelsa* might explain why traditional healers in the south-west region of Cameroon use the plant to cure back pain, toothache, stomach problems, and cough (Ndenecho, 2009). Nonetheless, previous reports described some flavonoids as antimicrobial (Mbaveng *et al*., 2012), analgesic, and anti-inflammatory secondary metabolites (Orhan *et al*., 2006). In continuation of the search for minor constituents from this plant, a new benzylic diglycoside has been identified along with four known compounds. We herein report on the structure elucidation of the new secondary metabolite.

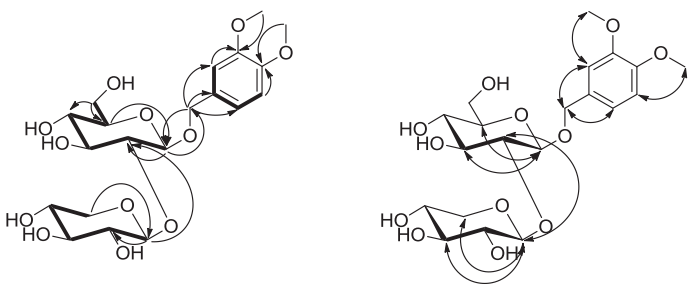
## Results and Discussion

Compound **1** was obtained as a colourless gum and gave in its HR-ESI mass spectrum a pseudomolecular peak at $m/z$ 485.1631 consistent with the molecular formula $[C_{20}H_{30}O_{12} + Na]^+$. This elemental composition corresponds to six double bond equivalents. Compound **1** responded positively to Molisch's reagent indicative for glycosides. This sug-

gestion was further supported by the presence of the resonances of two anomeric protons at $\delta$ 4.45 (d, $J = 7.7$ Hz)/101.6 ppm and 4.52 (d, $J = 7.5$ Hz)/106.3 ppm in the NMR spectra (Table I) as well as by a broad infrared absorption band at 3383 $cm^{-1}$ (OH). Furthermore, signals of an ABX spin system at $\delta$ 6.90 (d, $J = 8.2$ Hz)/112.5 ppm, 6.94 (dd, $J = 1.9, 8.2$ Hz)/121.8 ppm, and 7.10 (d, $J = 1.9$ Hz)/113.2 ppm were observed which suggest the presence of an electron-rich 1,3,4-trisubstituted arene. In addition to two diastereotopic benzylic oxymethylene resonances at $\delta$ 4.62 (d, $J = 11.5$ Hz) and 4.85 (d, $J = 11.5$ Hz)/71.5 ppm, signals of two methoxy groups at $\delta$ 3.82 (s)/56.4 ppm and 3.85 (s)/56.5 ppm were observed in the NMR spectra of **1**. The HMBC spectrum (Fig. 1) showed correlations supporting that these methoxy groups are attached to two neighbouring aromatic carbon atoms at $\delta_C$ 150.1 and 150.4 ppm, respectively. The first sugar moiety was connected to the benzylic methylene group since the protons of the latter showed correlations with one of the two anomeric carbon atoms ($\delta_C$ 101.6 ppm). The proton ($\delta_H$ 4.45 ppm) attached to that anomeric carbon atom correlated with a downfield oxygenated carbon atom at $\delta_C$ 83.8 ppm which in turn had the same interaction with the second anomeric proton at $\delta_H$ 4.52 ppm. This observation suggested the second sugar moiety to be connected to the rest of the molecule by the oxymethine group at $\delta_C$ 83.8 ppm adjacent to the carbon atom at $\delta_C$ 101.6 ppm (C-1′). Further correlations were found between the benzylic protons ($\delta_H$ 4.62 and 4.85 ppm) and carbon atoms at $\delta_C$ 131.8 (C-1), 113.2 (C-2), and 121.8 ppm

Table I. NMR data ($CD_3OD$, 600 MHz, $J$ in Hz) of compound **1** and $^{13}C$ NMR data of a compound previously reported by Sudo *et al*. (2000) (**A**, $CD_3OD$).

| Position | **1** | | **A** |
|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_C$ |
| 1 | – | 131.8 | 139.2 |
| 2 | 7.10 (1H, d, 1.9) | 113.2 | 129.0 |
| 3 | – | 150.4 | 129.3 |
| 4 | – | 150.1 | 128.6 |
| 5 | 6.90 (1H, d, 8.2) | 112.5 | 129.3 |
| 6 | 6.94 (1H, dd, 1.9, 8.2) | 121.8 | 129.0 |
| 7 | 4.62 (1H, d, 11.5), 4.85 (1H, d, 11.5) | 71.5 | 72.1 |
| 1′ | 4.45 (1H, d, 7.7) | 101.6 | 102.4 |
| 2′ | 3.44 (1H, dd, 7.7, 9.2) | 83.8 | 83.9 |
| 3′ | 3.53 (1H, *pseudo*-t, 9.0) | 78.0 | 77.9 |
| 4′ | 3.33 (1H, m) | 71.4 | 71.2 |
| 5′ | 3.25 (1H, m) | 77.9 | 77.9 |
| 6′ | 3.68 (1H, dd, 6.0, 12.0), 3.89 (1H, dd, 2.2, 12.0) | 62.7 | 62.8 |
| 1″ | 4.52 (1H, d, 7.5) | 106.3 | 106.3 |
| 2″ | 3.23 (1H, dd, 7.5, 9.0) | 75.7 | 75.9 |
| 3″ | 3.30 (1H, m) | 77.4 | 77.4 |
| 4″ | 3.46 (1H, m) | 71.1 | 71.5 |
| 5″ | 3.11 (1H, dd, 10.1, 11.6), 3.84 (1H, m) | 67.2 | 67.2 |
| MeO-3 | 3.85 (3H, s) | 56.5 | – |
| MeO-4 | 3.82 (3H, s) | 56.4 | – |




TOCSY (bold), HMBC (arrow) NOESY

Fig. 1. 2D NMR correlations of compound **1**.

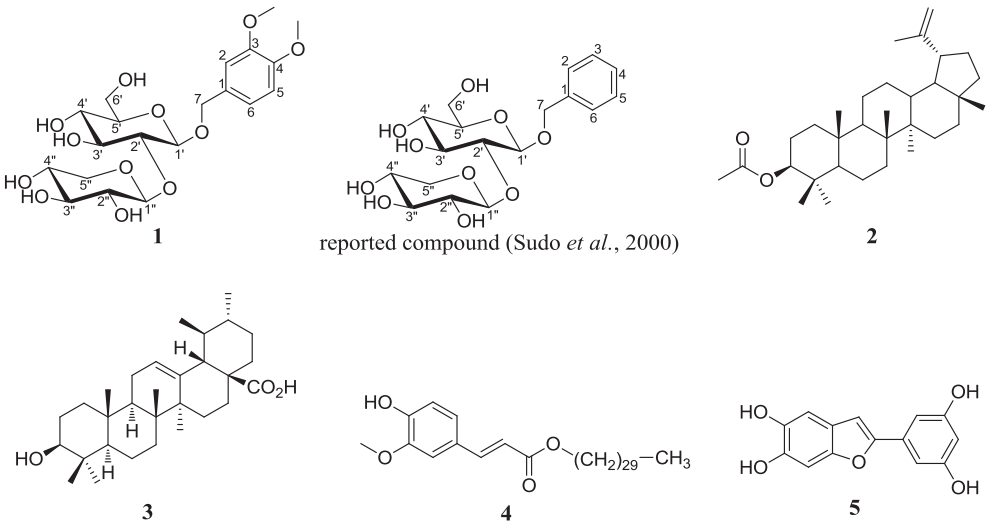



Fig. 2. Chemical structures of the isolated compounds 3,4-dimethoxybenzyl $\beta$-D-xylopyranosyl(1→2)-$\beta$-D-glucopyranoside (**1**), lupeol acetate (**2**), ursolic acid (**3**), triacontyl (*E*)-ferulate (**4**), and 2-(3,5-dihydroxyphenyl)benzofuran-5,6-diol (**5**).

(C-6). NOESY and TOCSY correlations (Fig. 1) permitted to deduce a pentopyranose and a hexopyranose structure for the terminal sugar moiety and the one attached to the aglycone, respectively. The coupling constants $J = 7.7$ and 7.5 Hz of their anomeric protons in conjunction with diaxial spatial correlations revealed in the NOESY spectrum (Fig. 1) suggested the terminal sugar to be $\beta$-xylopyranose and the internal one to be $\beta$-glucopyranose. While the proton at $\delta_H$ 4.45 ppm was spatially correlated to the axial protons at $\delta_H$ 3.53 and 3.25 ppm in the first sugar portion, the other resonance ($\delta_H$ 4.52 ppm) showed a similar spatial contact with axial protons at $\delta_H$ 3.11 and 3.30 ppm. Further evidence came from NMR data reported for related compounds (Kanho *et al.*, 2005; Sudo *et al.*, 2000) which confirmed the skeleton of both sugar moieties we assumed to be present in the common D-configuration. The chemical shifts of one of the compounds are given for comparison, *vide supra*. Accordingly, compound **1** was identified as 3,4-dimethoxybenzyl $\beta$-D-xylopyranosyl(1→2)-$\beta$-D-glucopyranoside (Fig. 2). The trivial name excelsoside was assigned.

In the course of this study, four known secondary metabolites were also obtained and identified as lupeol acetate (**2**) (Mahato and Kundu, 1994), ursolic acid (**3**) (Ayupbek *et al.*, 2012), triacontyl (*E*)-ferulate (**4**) (David *et al.*, 2004), and 2-(3,5-dihydroxyphenyl)benzofuran-5,6-diol (**5**) (Noguchi *et al.*, 1994) based on their NMR data.

## Conclusion

The secondary metabolites of *M. excelsa* can be divided into two groups including triterpenes and phenolic secondary metabolites (phenolic and benzylic glycosides, flavonoids, ferulates, and benzofurans). These plant metabolites might be responsible for the empirical bio-activities of the plant extracts against pain, toothache, and stomach problems as reported in ethnopharmacological studies (Ndenecho, 2009). Previous studies on similar metabolites supported this conclusion (Yoo *et al.*, 2009; Thirugnanasambantham *et al.*, 1990). Thus, salidroside, a phenolic glucopyranoside with certain resemblance to compound **1**, has been reported as an analgesic and antigastropathic compound (Yoo *et al.*, 2009).

## Experimental

### *Instrumentation*

Column chromatography (CC) and thin-layer chromatography (TLC) were performed over silica gel 0.035 – 0.070 mm (Merck, Darmstadt, Germany)/60 Å and $60F_{254}$, respectively. 1D- and 2D-NMR spectra were recorded on an AVANCE III-600 MHz spectrometer (Bruker, Karlsruhe, Germany) equipped with a 5-mm inverse TCI cryoprobe using standard pulse sequences. The IR spectrum was recorded on a Bruker Tensor 27 IR spectrometer equipped with a diamond ATR unit. The optical rotation was measured on

a Perkin Elmer model 241 polarimeter (Offenbach, Germany) at 546 and 578 nm and was extrapolated to 589 nm using Drude's equation. HR-ESIMS was carried out with a Q-ToF ULTIMA-III quadrupole TOF mass spectrometer (Waters, Eschborn, Germany). The UV spectrum was recorded on a Thermo Scientific evolution 201 UV-visible spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

*Plant material*

The leaves of *M. excelsa* were collected on June 28, 2011 in Yaoundé, Cameroon, and were identified by the staff of the national herbarium where a voucher specimen was deposited under the registration number HNC 57226.

*Extraction and isolation*

Air-dried leaves of *M. excelsa* (2 kg) were macerated in dichloromethane (DCM)/methanol (MeOH) (1:1), and the extract was concentrated *in vacuo* to afford a dark green organic residue (25 g). The crude extract was poured onto water and extracted successively with *n*-hexane (hex), ethyl acetate (EA), and *n*-butanol (*n*-BuOH) yielding three fractions, A (8 g), B (15 g), and C (1 g), respectively. Fraction C was subjected to repeated silica gel CC eluted with DCM/MeOH in gradient conditions giving 78 sub-fractions. Compound **1** (4.5 mg) precipitated in acetone from sub-fractions 35 – 40 [eluted with DCM/MeOH (85:15)]. Fraction A was further purified using silica gel CC eluted with gradients of hex/EA to yield 60 sub-fractions. Compound **2** (lupeol acetate; 3.1 mg) was filtered from sub-fractions 15 – 18 eluted with hex/EA (9:1). Compound **4** [triacontyl (*E*)-ferulate; 3.1 mg] was obtained from sub-fractions 40 – 42 eluted with hex/EA (7:3). Fraction B was purified in the same manner as the abovementioned fractions and was eluted with gradients of DCM/MeOH. This work provided 120 sub-fractions, and compound **3** (ursolic acid; 4.3 mg) was obtained from sub-fractions 3 – 8 eluted with DCM. Compound **5** [2-(3,5-dihydroxyphenyl)benzofuran-5,6-diol; 4.0 mg] precipitated in sub-fractions 30 – 33 eluted with DCM/MeOH (98:2).

*3,4-Dimethoxybenzyl β-D-xylopyranosyl(1→2)-β-D-glucopyranoside (excelsoside)* (**1**): Colourless gum. – $[\alpha]_D^{20}$ −16.6 (*c* 0.093, $CD_3OD$). – UV (MeOH): $\lambda_{max}$ (log $\varepsilon$) = 204 (5.31), 229 (4.77), 278 (4.12) nm. – IR (ATR) $\nu$ = 3383, 2920, 1516, 1464, 1265, 1077, 1044 $cm^{-1}$. – $^1H$ and $^{13}C$ NMR: see Table I. – HR-ESI-MS: $m/z$ = 485.1631 (calcd. for $[C_{20}H_{30}O_{12} + Na]^+$, 485.1635).

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