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Triterpenoid saponins from *Pteleopsis suberosa* stem bark

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Dedicated to the memory of Prof. Ivano Morelli.

Abstract

Thirteen oleanane saponins (**1**–**13**), four of which were new compounds (**1**–**4**), were isolated from *Pteleopsis suberosa* Engl. et Diels stem bark (Combretaceae). Their structures were determined by 1D and 2D NMR spectroscopy and ESI-MS spectrometry. The compounds were identified as 2α,3β,19α,23,24-pentahydroxy-11-oxo-olean-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**1**), 2α,3β,19β,23,24-pentahydroxy-11-oxo-olean-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**2**), 2α,3β,19α,23-tetrahydroxy-11-oxo-olean-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**3**), and 2α,3β,6β,19α,24-pentahydroxy-11-oxo-olean-12- en-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**4**). The presence of α,β-unsaturated carbonyl function was not common in the oleanane class and the aglycons of these compounds were not found previously in the literature. Moreover, the isolated compounds were tested against *Helicobacter pylori* standard and *vacA*, and *cagA* clinical virulence genotypes. Results showed that compound **6** has an anti-*H. pylori* activity against three metronidazole-resistant strains (Ci 1 *cagA*, Ci 2 *vacA*, and Ci 3).

Keywords: *Pteleopsis suberosa*; Combretaceae; *Helicobacter pylori*; Triterpenoid saponins

1. Introduction

In the course of our investigations on plants belonging to Malian flora, the chemical composition of *Pteleopsis suberosa* Engl. et Diels bark has been studied. The genus *Pteleopsis* belongs to Combretaceae family which includes 20 genus and about 600 species of plants distributed especially in the Tropical and Sub-Tropical regions, and in West Africa (Hutchinson, 1959). *P. suberosa* is a small tree quite widespread in the savanna and in African regions as Mali, Senegal, Guinea, Ghana, Togo, Benin, and Nigeria. The leaves of *P. suberosa* are popularly known for the treatment of meningitis, convulsive fever, and headache, while the bark is reported able to increase cereal productivity (Kerharo and Adam, 1974) and to treat jaundice, asthenia, and dysentery (Adjanhoun et al., 1986). In the Malian folk medicine the stem bark, commonly named "terenifù", is known as a traditional remedy against cough, asthma, hemorrhoids, virus, and especially against ulcer (Mariko, 1989). In the Malian Pharmacopea, the powdered bark constitutes the Calmogastryl®, a Traditional Improved Drug orally administered as decoction for the treatment of gastric and duodenal ulcers. Extracts obtained from the bark demonstrated fungicidal and fungistatic activity (Baba-Moussa et al., 1999), antitussive properties (Occhiuto et al., 1999), and antiulcer action (De Pasquale et al., 1995). Moreover, the methanolic extract showed an *in vitro* anti-*Helicobacter pylori* activity (Germanò et al., 1998). Previous phytochemical studies on the genus *Pteleopsis* were performed only on

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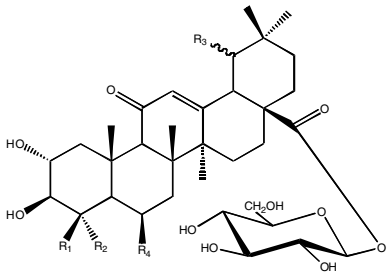
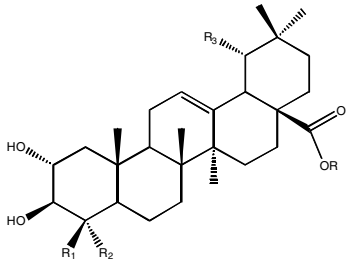
the species *P. hylodendron*, reporting the isolation and elucidation of two oleanane saponins (Ngounou et al., 1999). The aim of our work was to carry out the chemical study of *P. suberosa* bark never reported before; herein we describe the isolation and structural characterization of 13 triterpenoids (**1**–**13**), four of which (**1**–**4**) were new triterpenoid glycosides, together with their anti-*Helicobacter pylori* activities against standard and *vacA* and *cagA* clinical virulence genotypes.



Compound	R_1	R_2	R_3	R_4
1	-CH_2OH	-CH_2OH	αOH	-H
2	-CH_2OH	-CH_2OH	βOH	-H
3	-CH_3	-CH_2OH	αOH	-H
4	-CH_2OH	-CH_3	αOH	-OH



Compound	R	R_1	R_2	R_3
5	Glc	-CH_2OH	-CH_2OH	-OH
6	Glc	-CH_3	-CH_2OH	-OH
7	Glc	-CH_2OH	-CH_3	-OH
8	Glc	-CH_3	-CH_3	-OH
9	Glc	-CH_3	-CH_2OH	-H
10	Glc	-CH_2OH	-CH_2OH	-H
11	-H	-CH_2OH	-CH_3	-OH
12	-H	-CH_3	-CH_2OH	-OH
13	-H	-CH_2OH	-CH_2OH	-OH

Glc = β-D-glucopyranoside

2. Results and discussion

The chemical study of *n*-butanol and chloroform-methanol extracts of *P. suberosa* bark, by different chromatographic techniques, afforded 13 triterpenoid compounds, including 10 saponins and 3 aglycons. Compounds **1**–**4** resulted new natural products and were identified by 1D, 2D NMR spectroscopy, and ESI-MS analyses while the others compounds are known triterpenes identified by comparison of their NMR data with those reported in the literature as trachelosperidoside E-1 (**5**) (Fumiko and Tatsuo, 1987), arjunglucoside I (**6**) (Zhou et al., 1992), sericoside (**7**) (Terreaux et al., 1996), arjunetin (**8**) (Braca et al., 2001), arjunglucoside II (**9**) (Jayasinghe et al., 1993), bellericoside (**10**) (Nandy et al., 1989), sericic acid (**11**) (Bombardelli et al., 1974), arjungenin (**12**) (Jossang et al., 1996), and trachelosperogenin (**13**) (Mahato et al., 1992).

Compound **1** had a molecular formula $C_{36}H_{56}O_{13}$, as determined by ^{13}C, ^{13}C-DEPT NMR, positive ESI-MS spectrum (quasi-molecular ion peak at *m/z* 719 $[M+Na]^+$), and elemental analysis. In the ESI-MS spectrum of **1** was also evident a fragment at *m/z* 557 $[M+Na\text{-}162]^+$, due to the loss of a hexose unit. The ^{13}C NMR data (see Table 1) of compound **1** showed the presence of 36 signals, 30 of which were assigned to a triterpenoid moiety and 6 to the saccharide portion. The UV spectrum of **1** (λ_{max} at 255 nm) suggested the presence of α,β-unsaturated carbonyl function which was confirmed by carbon signals in the ^{13}C NMR spectrum at δ 128.9 (C-12), 174.0 (C-13), and 203.0 (C-11) whose resonances are consistent with references reported in the literature for 11-oxo-oleanolic aglycon type (Ikuta et al., 1995). Also the downfield shift (+4 ppm) of olefinic carbon C-12 (δ 128.9) is consistent with presence of conjugated carbonyl group (Seebacher et al., 2003). The ^1H NMR spectrum (Table 1) exhibited also the presence of signals of five tertiary methyl groups (δ 0.95, 0.98, 0.99, 1.20, and 1.58), while the resonances at δ 3.40 (*d*, $J = 5.0$ Hz), 3.50 (*d*, $J = 10.0$ Hz), and 3.91 (*ddd*, $J = 12.0$, 10.0, 3.0 Hz) were due to three protons linked at carbons bearing one hydroxyl group. The relative configurations of the hydroxylated carbons were assigned as 2α, 3β, and 19α mainly on the basis of ^1H NMR coupling and by comparison with those reported for related compounds (Fumiko and Tatsuo, 1987). We also noted that the presence of the OH group at the 19 position induced a downfield shift of the resonance of the axial proton H-16 (δ 2.47, *ddd*, $J = 13.0$, 13.0, 4.5 Hz), thus supporting the 19α-OH stereochemistry and being compatible only with a *cis* stereochemistry of the ring D/E junction. The observation of two AB doublets (δ 3.56 and 4.05, $J = 9.5$ Hz; δ 3.70 and 4.08, $J = 11.0$ Hz) indicated the presence of two hydroxymethylene. Assignments of all chemical shifts of protons and carbons of aglycon portion were ascertained from a combination of 1D-TOCSY, DQF-COSY, and HSQC spectral analysis. The substitution sites on the triterpene skeleton were confirmed by HMBC experiment showing correlation peaks between H-12 and C-14, C-9, and C-11, demonstrating the 11,12 position of enone group; between Me-30 and C-20, C-19, and C-21, consistent with the presence of a hydroxy group at C-19; finally, between H-23a and C-4, C-24, and C-3, in agreement with all the assignments of A ring. Identification of the saccharide unit was performed by analysis of NMR data: the chemical shifts, the multiplicity of signals, and the values of the coupling constants were in agreement with