Five new iridoïd dimers from the fruits of Canthium subcordatum DC (syn. Psydrax subcordata DC)

Christelle Joubouhi, Florence Déclaire Mabou, Perrin Lanversin Foning Tebou, David Ngnokam, Dominique Harakat, Laurence Voutquenne-Nazabadioko

Five new iridoïd dimers from the fruits of *Canthium subcordatum* DC (syn. *Psydrax subcordata* DC)

Christelle JOUBOUHI [a], Florence Déclaire MABOU[a], Perrin Lanversin FONING TEBOU[a], David NGNOKAM[a*], Dominique HARAKAT[b] and Laurence VOUTQUENNE-NAZABADIOKO[c]

[a]Faculty of Science, Department of Chemistry, University of Dschang, P.O. Box 67. Dschang Cameroon

[b]Service Commun d'Analyses, Institut de Chimie Moléculaire de Reims (ICMR), CNRS UMR 7312, Bat. 18 B.P.1039, 51687 Reims Cedex2, France

[c]Groupe Isolement et Structure, Institut de Chimie Moléculaire de Reims (ICMR), CNRS UMR 7312, Bat. 18 B.P.1039, 51687 Reims Cedex2, France

[*]Corresponding author. Tel. (237)696710992. E-mail. dngnokam@yahoo.fr

Abstract

Five new iridoid dimmers, canthiumosides 1-5 (**1-4** and **5a**), together with nine known compounds, shanzhigenin methyl ester (**6),** 1-epishanzhigenin methyl ester **(6'**), linearin (**7),** 1-epilinearin (**7'**), mussaenoside (**8**), shanzhiside methyl ester (**9**), 3',4',7- trihydroxyflavone (**10**), betulinic acid (**11**), and oleanolic acid (**12**) were isolated from the fruits of *Canthium subcordatum* DC (Syn. *Psydrax subcordata* (DC) Bridson). The structures of these compounds were established by interpretation of their spectral data, mainly HR-TOFESIMS, 1D-NMR (^{1}H, ^{13}C and DEPT) and 2D-NMR (^{1}H-^{1}H COSY, HSQC, HMBC, and NOESY), and by comparison with the literature.

Keywords: *Canthium subcordatum/Psydrax subcordata*; Rubiaceae; iridoid dimers; canthiumoside; structure elucidation.

1. Introduction

Canthium subcordatum (formely *Psydrax subcordata*) is a tree which grows in central and western Africa and reaches a height of more than 10 m (Irvine, 1961). Its roots, leaves and stem bark are used for medicinal purposes (Ampofo, 1977). Alcoholic extracts of the stem bark have potential antidiabetic properties (Ampofo, 1977) and the roots are used to treat malaria fever, inflammation and cardiovascular disease (Awah et al., 2012). Previous work on this genus revealed the presence of iridoids (Achenbach et al., 1980, 1981), peptidic alkaloids (Dongo al., 1989; Achenbach et al., 1986), terpenoids and miscellaneous compounds (Patro et al., 2014). In the course of our continuing search for secondary metabolites of biological importance from Cameroonian medicinal plants, we investigated the *iso*BuOH, EtOAc and *n*-hexane extracts of the fruits of *C. subcordatum*. In the present paper, we report the isolation and structural elucidation of five new iridoid dimers using chemical and spectroscopic methods.

2. Results and Discussion

Purification of the *iso*BuOH, EtOAc and *n*-hexane soluble fractions of the crude MeOH extract afforded five new compounds, canthiumosides 1-5 (**1-4** and **5a)**, and nine known compounds (**6-12**). The structures of the known compounds were determined by means of co-TLC and by comparative analysis of their physical and spectral data with those reported in the literature for shanzhigenin methyl ester (**6)** and 1-epishanzhigenin methyl ester (**6'**) (Guo et al., 2001), linearin (**7)** and 1-epilinearin (**7'**) (Khatri et al., 1979), mussaenoside (**8**) (Takeda et al., 1997), shanzhiside methyl ester (**9**) (Takeda et al., 1997), 3',4',7-trihydroxyflavone (**10**) (Bickoff et al., 1965), betulinic acid (**11**) (Sholichin et al., 1980), and oleanolic acid (**12**) (Hossain et Ismail, 2013) (Figure 1).

Compound **1** (canthiumoside 1) was obtained as a waxy solid. Its HR-TOFESIMS exhibited a pseudo-molecular ion peak at *m/z* 485.1428 $[M+Na]^+$ (calcd. for $C_{23}H_{26}O_{10}Na$. 485.1424), indicating the molecular formula $C_{23}H_{26}O_{10}$ with twelve degrees of unsaturation. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) showed mainly doubled peaks suggesting that compound **1** was an iridoid dimer. The two iridoid moeities are hereafter referred to as units a and b. The proton and carbon signals of these two units closely resembled those of the ulmoidosides (Yahara et al., 1990). The ^1H NMR spectrum showed four olefinic protons at δ_H 7.55 (H-3a, s), δ_H 7.61 (H-3b, s) and δ_H 5.91 (H-7a and b, br s); four methylene protons at δ_H 2.06 (H-α-6a and b, m) and δ_H 2.87 (H-β-6a and b, m); a methoxy group at δ_H 3.73 (11a-OMe, s); six methine protons at δ_H 4.84 (H-1a, d, J = 8.6 Hz), 4.82 (H-1b, d, J = 8.6 Hz), 3.18 (H-5a q, J =

8.5 Hz), 3.21 (H-5b, q, J = 8.5 Hz) and δ_H 2.56 (H-9a and b, t, J = 8.5 Hz); two oxymethylenes at δ_H 4.91 (H-10a, d, J = 14.4 Hz), δ_H 4.78 (H-10a, d, J = 14.4 Hz), 4.81 (H-10b, d, J = 14.5 Hz) and δ_H 4.76 (H-10b, d, J = 14.5 Hz) attributable to an iridoid framework (Patro et al., 2014; Cui-lian et al., 2010; Demirezer et al., 2006) as summarized in Table 1. This was confirmed by the ^{13}C NMR spectrum, which exhibited 23 carbon signals due to 10 pairs of carbons of the main iridoid skeleton (Quang et al., 2002), including two *α,β*-unsaturated ester groups at *δ*c 168.4 (C-11a) and *δ*c 167.6 (C-11b), eight olefinic carbons at *δ*c 153.2 (C-3b), 152.9 (C-3a), 110.2 (C-4a and b), 139.3 (C-8a), 139.1 (C-8b), 129.3 (C-7a) and *δ*c 129.7 (C-7b); an acetyl group at *δ*c 171.3 (C-10b-$\underline{C}OCH_3$) and δ_C 19.4 (C-10b-CO$\underline{C}H_3$) and a methoxyl carbon at δ_C 50.3 (11a-OMe) (Table 2). The two hemiacetal protons at δ_H 4.82 (H-1b) and δ_H 4.84 (H-1a) correlated in the HSQC spectrum with carbons at δ_C 96.3 (C-1b) and 96.2 (C-1a), respectively. Comparison of these chemical shifts with those of linearin (Guo et al., 2001) and shanzhigenin methyl ester (Khatri et al., 1979) indicated that the two hydroxy groups at C-1a and C-1b were not involved in any ether bond.

In the COSY spectrum, protons at δ_H 4.82 (H-1b) and 4.84 (H-1a) correlated with protons at δ_H 2.56 (2H, t, J = 8.5 Hz) attributed to H-9a and H-9b, which correlated to protons at δ_H 3.18 (H-5a, q, J = 8.5 Hz) and δ_H 3.21 (H-5b, q, J = 8.5 Hz). In addition these two protons showed cross peaks with protons at δ_H 2.06 (H-6αa and H-6αb, m) and δ_H 2.87 (H-6βa and H-6βb, m) which correlated with the olefinic protons at δ_H 8.91 (H-7a, H-7b). The corresponding carbons of these protons were assigned by analysis of the HSQC spectrum (Table 1). In the HMBC spectrum the vinyl protons H-7a and H-7b showed cross-peaks with carbons at δ_C 139.3 (C-8a), 139.1 (C-8b), 38.8 (C-6a), 38.7 (C-6a), 35.9 (C-5a), 36.0 (C-5b) and with the hemiacetal carbons C-1a and C-1b. The protons at δ_H 4.78 and 4.91 (H-10a, d, J = 14.4 Hz), showed long-rang correlations with carbon C-7a (*δ*c 129.3). Similarly, protons 2H-10b (δ_H 4.76 and 4.81, d, J = 14.5 Hz) showed long-rang correlations with carbon C-7b (δ_C 127.9). The presence of cross-peaks between 2H-10b and the carbonyl carbon at δ_C 171.2 revealed the attachment of the acetate to C-10b (Patro et al., 2014; Yahara et al., 1990; Cui-Lian et al., 2010).

In the HMBC spectrum, vinyl proton H-3a (δ_H 7.55) correlated with C-1a (δ_C 96.2), C-4a (δ_C 110.1), C-11a (δ_C168.3) and C-5a (*δ*C 35.9) while vinyl proton H-3b at δ_H 7.61 showed correlations with C-1b (*δ*c 96.3), C-4b (δ_C 110.2), C-11b (δ_C 167.6) and C-5b (δ_C 36.0). The position of the methoxy group (δ_C 50.3) was revealed by a cross-peak between the methoxyl protons at (δ_H 3.73) and the carbonyl carbon at δ_C168.3 (C-11a). The position of the ester

linkage between units a and b was determined by the presence of cross-peaks between protons 2H-10a (δ_H 4.91 and 4.78) and the carbonyl carbon C-11b (δ_C 167.6) (Sui-Kiong et al., 2002) Confirmation of the stereochemistry of the stereogenic centers was achieved by analysis of *J* values and comparison of ^{13}C chemical shifts with literature data, especially those for the chiral centers at C-5a, C-9a and C-5b, C-9b, which indicated the beta-configuration of H-9 as in geniposide (Inouye et al., 1974; Bailleul et al., 1977). The data were also consistent with the normal *cis* junction between the two rings and the beta-configurations of the hydroxyl residue at C-1 (Hamerski et al., 2003; Zapesochnaya et al., 1991). This was confirmed by NOESY correlations between H-5 and H-9, as well as H-6β, in both units. Thus structure **1** was assigned to the iridoid dimmer canthiumoside 1 (Figure 1).

Compound **2** was obtained as a waxy solid. Its HR-TOFESIMS exhibited a pseudo-molecular ion peak at *m/z* 647.1946 $[M+Na]^+$ (calcd. for $C_{29}H_{36}O_{15}Na$. 647.1952), indicating a molecular formula $C_{29}H_{36}O_{15}$ with thirteen degrees of unsaturation. The ^1H and ^{13}C-NMR spectra (Tables 1 and 2) displayed two sets of signals typical of a dimeric iridoid and showed similar chemical shifts to those of compound **1**, apart from additional signals for a glucose moiety at δ_H 4.74 (H-1'b, d, *J* = 7.8 Hz), 3.24 (H-2'b, dd, *J* = 9.1, 7.8 Hz), 3.40 (H-3'b, t, *J* = 9.1 Hz), 3.31 (H-4'b, m), 3.31 (H-5'b, m), 3.67 (H-6'b, dd, *J* = 11.7, 5.8 Hz), 3.89 (H-6'b, dd, *J* = 11.7, 1.7 Hz) and at δ_C 99.0 (C-1'b), 73.4 (C-2'b), 76.5 (C-3'b), 70.1 (C-4'b), 77.0 (C-5'b) and 61.4 (C-6'b). Analysis of COSY and HSQC spectra revealed the presence of a glucopyranose moiety (Patro et al., 2014; Cui-Lian et al., 2010) (Tables 1 and 2) characterized by coupling constants up to 7 Hz for protons H-2', H-3' and H-4'. The anomeric configuration of the glucose was determined to be beta from the $J_{H\text{-}1'\text{-}H\text{-}2'}$ = 7.5 Hz (Kanchanpoom et al., 2002). Acid hydrolysis followed by column chromatography over silica gel with MeCN/H_2O as eluent, afforded D-glucose.

The linkage of the glucopyranosyl moiety to C-1b of the aglycone was clearly indicated by the cross-peak between the anomeric carbon (δ_C 99.0, C-1'b) and H-1b (δ_H 5.22, d, *J* = 7.7 Hz). Compound **2** afforede the hexa-acetate **2a** on acetylation, confirming the presence of a free hydroxyl group at C-1a. Thus structure **2** was assigned to canthiumoside 2 (Figure 1).

Compound **3,** a waxy solid, had a molecular formula $C_{35}H_{46}O_{20}$, deduced from the pseudo-molecular ion peak at *m/z* 809.2477 $[M+Na]^+$ (calcd. for $C_{35}H_{46}O_{20}Na$. 809.2480) in its HR-TOFESIMS spectrum. The ^1H and ^{13}C-NMR spectra (Tables 1 and 2) displayed two sets of signals typical of a dimeric iridoid and showed similar chemical shifts to those of compound **2**, except for the presence of signals of a second hexose moiety. A *β*-glucopyranose moiety was identified starting from the anomeric proton at δ_H 4.73 (H-1'a, d, *J* = 7.8 Hz) in the

COSY spectrum (Patro et al., 2014; Cui-Lian et al., 2010) and confirmed by the carbon chemical shifts (Kanchanpoom et al., 2002). Again acid hydrolysis afforded only *D*-glucose.
The attachment of the second glucopyranose moiety to C-1a was clearly indicated by the cross-peak between the anomeric carbon (δ_C 98.9, C-1'a) and H-1a at δ_H 5.24. In addition, comparison of the ^{13}C data of **3** with those of related compounds (Zeng et al., 2010) supported structure **3** for canthiumoside 3 (Figure 1).
Compound **4** was obtained as a waxy solid. Its HR-TOFESIMS exhibited a pseudo-molecular ion peak at *m/z* 767.2369 $[M+Na]^+$ (calcd. for $C_{33}H_{44}O_{19}Na$. 767.2374), indicating that the molecular formula $C_{33}H_{44}O_{19}$ and twelve degree of unsaturation. The ^1H and ^{13}C NMR spectra were very similar to those of **3**, except the absence of the acetyl group at C-10b. Acid hydrolysis again afforded only *D*-glucose. Structure **4** for canthiumoside 4 (Figure 1) was also supported by the literature data of related compounds (Zeng et al., 2010).

Compound **5** was obtained pure only as its acetate **5a** and its structure elucidation was carried out with the acetate. However, NMR spectra of the mixture containing the compound **5** were in complete agreement with the proposed structure. The general appearance of the ^1H and ^{13}C NMR spectra of the mixture suggested that **5** was an iridoid dimmer with a glycosyl moiety. The ^1H NMR spectrum contained three hemiacetal proton doublets whose HMBC correlations were also consistent with a dimeric iridoid skeleton. Comparison with NMR data of compounds **2** and **3** showed that compound **5** possessed the same unit a glucosylated at C-1a but differed in the structure of unit b. The HR-TOFESIMS of the pentaacetate **5a** showed a pseudo-molecular ion peak at *m/z* 801.2592 $[M+Na]^+$ (calcd. 801.2582) corresponding to the elemental composition $C_{37}H_{46}O_{18}$. This was consistent with the ^{13}C NMR spectrum which displayed thirty seven carbons (Table 2). The ^1H NMR spectrum contained six methyl signals, five of which at δ_H 2.15, 2.11, 2.05, 2.02 and 1.99 were due to acetates. The structure of **5a** was confirmed by analysis of the 2D NMR (COSY, HSQC and HMBC) data. A beta-glucopyranose moiety was identified in the COSY spectrum starting from the anomeric proton at δ_H 4.90 (H-1'a, J = 7.9 Hz). The expected HMBC correlations confirmed that unit a, with a glucopyranose unit attached to C-1a, was the same as in compounds **1**-4 and was connected to unit b *via* the same ester link. In the COSY spectrum, H-1b at δ_H 6.05 (d, J = 7.7 Hz) correlated with H-9b (δ_H 2.25) which, in turn, showed a cross-peak with H-8b (δ_H 2.35). A further cross-peak from H-8b to a methyl group Me-10b (δ_H 1.05, d, J = 5.1 Hz) revealed the structure of unit b. The assignments of all the protons and carbons of unit B followed readily from analysis of the HMBC spectrum (Tables 1 and 2). The relative stereochemistry

of **5a** was supported by its NOESY spectrum which showed correlations between H-5bβ and H-9bβ, and between Me-10b and H-1bα showing that the methyl group is alpha. Comparison of the ^{13}C data of **5a** with those of related compounds S(Zhang et al., 2009) supported structure **5** for canthiumoside 5 (Figure 1).

3. Experimental

3.1. General

The melting points were recorded with a Reichert microscope (Reichert Technologies, Depew, New York USA) and are uncorrected. IR spectra were recorded with a Shimadzu FT-IR-8400S (Shimadzu, France) spectrophotometer. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded on a BRUKER Avance DRX-500 spectrometer (Bruker, Wissembourg, France) equipped with a BBFO+5 mm probe. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectra were recorded on a BRUKER Avance III-600 spectrometer (Bruker, Wissembourg, France) equipped with a cryoplatform using CD_3OD with TMS as the internal standard. TOF-ESIMS and HR-TOF-ESI experiments were performed using a Micromass Q-TOF micro instrument (Manchester, UK) with an electrospray source. The samples were introduced by direct infusion in a solution of MeOH at a rate of 5µl min^{-1}. The optical rotations were measured on a Bellingham & Stanley ADP 220) polarimeter (Bellingham + Stanley Ltd, United King-Dom). Column chromatography was run on Merck silica gel (VWR, France) 60 (70-230 mesh) and gel permeation on Sephadex LH-20 (VWR, France), while TLC was carried out on silica gel GF_{254} pre-coated plates with detection accomplished by spraying with 50% H_2SO_4 followed by heating at 100 °C or by visualizing with a UV lamp at 254 and 365 nm.

3.2. Plant material

The fruits of *Canthium subcordatum* DC (syn. *Psydrax subcordata* DC Bridson) were collected in Foto village (Menoua Division, Western region of Cameroon), in April 2012. Authentication was performed by Victor Nana, a botanist of the Cameroon National Herbarium, Yaoundé, where a voucher specimen (N° 19579/SRF/CAM) has been deposited.

3.3. Extraction and isolation

The dried fruits of *C. subcordatum* (3.5 kg) were extracted with MeOH at room temperature for 3 days, and the extract was concentrated to dryness under reduced pressure (130 g). Part of the residue (122 g) was suspended in water and successively extracted with *n*-hexane, ethyl acetate and *iso*butanol to obtain, after evaporation of solvent, 53.8 g, 6.6 g and 35 g respectively. Part of the *iso*butanol-solute extract (32 g) was subjected to silica gel CC eluting with EtOAc containing increasing MeOH (0%, 5%, 10%, 15%, 20% and 30%, each

500 ml) to give eight sub fractions (A to H). Fraction D (6.6 g) was purified by silica gel CC eluting with an EtOAc-MeOH (5%) mixture to give compound **8** (40 mg). Fraction H (8 g) was purified by silica gel CC eluting with an EtOAc-MeOH (15%) mixture to give compounds **4** (39 mg) and **3** (42 mg). G (1.4 g) was purified by silica gel CC eluting with an EtOAc-MeOH (15%) mixture to give compound **9** (36 mg). Fractions A to C were combined with the EtOAc-soluble extract (8.6 g) and the whole was eluted with *n*-hexane containing increasing EtOAc (0%, 5%, 10%, 15%, 20%, 30% and 70%), EtOAc and EtOAc-MeOH (5%) mixtures. Eight sub fractions, 1-8, were obtained. Fraction 5 (1.5 g) was purified by silica gel CC eluting with a Hex-EtOAc (15%) mixture to give compounds **1** (24 mg) and **7** (35 mg). Fraction 6 (430 mg) was purified by silica gel CC eluting with a Hex-EtOAc (80%) mixture to give compound **2** (36 mg). Fraction 7 (2 g) was purified by silica gel CC eluting with a *n*-Hex-EtOAc (40%) mixture to give compounds **10** (22 mg), **6** and **6'** (32 mg). Fraction 8, containing compound **5**, could not be purified and was acetylated (acetic anhydridre:pyridine 1:1). The pentaacetate **5a** was isolated by silica gel CC. Part of *n*-hexane-solute extracts (50 g) was eluted with *n*-hexane containing increasing EtOAc (0%, 10%, 20%, 30%, 60%), EtOAc and EtOAc-MeOH (5%) mixtures to give five sub fractions A to E. The portion eluted with 20% ethyl acetate in *n*-hexane (fraction C) was chromatographed over silica gel with the same system to give compounds **11** (50 mg) and **12** (40 mg).

3.4. New Compound Data

Canthiumoside 1 **1.** Waxy solid, $[\alpha]_D^{23}$ - 43.2 (*c* 0.50, MeOH). IR (NaCl) ν_{max} (cm^{-1}):3500; 1754; 1640; 1602. ^1H (500 MHz) and ^{13}C-NMR (125 MHz) data in CD_3OD see Tables 1 and 2. HR-TOFESIMS *m/z*: 485.1428 $[M+Na]^+$ (calcd. for $C_{23}H_{26}O_{10}Na$, 485.1424)

Canthiumoside 2 **2.** Waxy solid, $[\alpha]_D^{23}$ – 17.6 (*c* 0.50, MeOH). IR (NaCl) ν_{max} (cm^{-1}):3540; 1744; 1643; 1622. ^1H (600 MHz) and ^{13}C-NMR (150 MHz) data in CD_3OD, see Tables 1 and 2. HR-TOFESIMS *m/z*: 647.1946 $[M+Na]^+$ (calcd. for $C_{29}H_{36}O_{15}Na$, 647.1952)

Canthiumoside 3 **3.** Waxy solid, $[\alpha]_D^{23}$ -0.6 (*c* 0.46, MeOH). IR (NaCl) ν_{max} (cm^{-1}):3500; 1754; 1630; 1612. ^1H (500 MHz) and ^{13}C-NMR (125 MHz) data in CD_3OD, see Tables 1 and 2. HR-TOFESIMS *m/z*: 809.2477 $[M+Na]^+$ (calcd. for $C_{35}H_{46}O_{20}Na$, 809.2480)

Canthiumoside 4 **4**. Waxy solid, $[\alpha]_D^{23}$ -3.9 (*c* 0.55, MeOH). IR (NaCl) ν_{max} (cm^{-1}):3550; 1750; 1645; 1610. ^1H (500 MHz) and ^{13}C-NMR (125 MHz) data in CD_3OD, see Tables 1 and 2. HR-TOFESIMS *m/z*: 767.2369 $[M+Na]^+$ (calcd. for $C_{33}H_{44}O_{19}Na$, 767.2374)

Canthiumoside 5a **5a**. Waxy solid, $[\alpha]_D^{23}$ –11.2 (*c* 0.50, $CHCl_3$). IR (NaCl) ν_{max} (cm^{-1}):1754; 1640; 1602. ^1H (600 MHz) and ^{13}C-NMR (150 MHz) data in $CDCl_3$, see Tables 1 and 2. HR-TOFESIMS *m/z*: 801.2592 $[M+Na]^+$ (calcd. for $C_{37}H_{46}O_{18}Na$, 801.2582)

3.6. Acetylation of 2, 6 and 6', 7 and 7'

Compound **2** (13 mg) was acetylated with Ac_2O–pyridine and the reaction mixture was purified over a silica gel column, eluting with a Hex-EtOAc (20%) mixture to give compound **2a** (Waxy solid, 6 mg). $[\alpha]_D^{23}$ -14.1 (*c* 0.58, $CHCl_3$). IR (NaCl) ν_{max} (cm^{-1}): 1754; 1640; 1602. HR-TOFESIMS *m/z*: 857.2488 $[M+Na]^+$ (calcd. for $C_{39}H_{46}O_{20}Na$, 857.2480). ^1H NMR ($CDCl_3$, 600 MHz) δ_H: 7.91 (1H, s, H-3b), 7.48 (1H, s, H-3a), 5.94 (1H, d, 6.9, H-1a), 5.92 (1H, br s, H-7a), 5.92 (1H, br s, H-7b), 5.88 (1H, d, 7.4, H-1b), 5.25 (1H, t, 9.3, H-3'b), 5.15 (1H, dd, 9.5, 9.3, H-4'b), 5.05 (1H, dd, 8.1, 9.3, H-2'b), 4.90 (1H, d, 8.1, H-1'b), 4.86 (1H, d, 13.5, H-10b), 4.85 (1H, d, 13.7), 4.72 (1H, d, 13.7, H-10a), 4.70 (1H, d, 13.5, H-10b), 4.27 (1H, dd, 13.0, 2.4), 4.16 (1H, dd, 13.0, 5.9, H-6'b), 3.75 (3H, s, C-11a -OMe), 3.75 (1H, m, H-5'b), 3.30 (1H, m, H-5a), 3.23 (1H, m, H-5b), 2.90 (1H, m, H-6-β-a), 2.89 (1H, t, 7.4, H-9b), 2.88 (1H, t, 8.2, H-9a), 2.87 (1H, m, H-6-β-b), 2.20 (1H, m, H-6-α-a), 2.18 (3H, s, C-1a-OAc). ^{13}C NMR ($CDCl_3$, 150 MHz) δ_C: 170.6 (C-10b-OAc), 170.5 (C-6'-OAc), 170.2 (C-3'-OAc), 169.4 (C-4'-OAc), 169.3 (C-1a-OAc), 169.1 (C-2'-OAc), 167.2 (C-11a), 166.4 (C-11b), 151.6 (C-3a), 151.4 (C-3b), 137.2 (C-8b), 136.6 (C-8a), 132.3 (C-7a), 130.8 (C-7b), 111.7 (C-4b), 111.3 (C-4a), 96.9 (C-1'b), 96.1 (C-1b), 91.5 (C-1a), 72.4 (C-3'), 72.0 (C-5'b), 70.7 (C-2'b), 68.2 (C-4'b), 62.5 (C-10b), 61.6 (C-6'b), 61.5 (C-10a), 51.4 (C-11a-OMe), 46.7 (C-9b), 45.3 (C-9a), 38.6 (C-6b), 38.4 (C-6a), 34.4 (C-5a), 34.0 (C-5b), 20.7 (C-4'-OAc; C-6'-OAc), 20.6 (C-3'-OAc), 20.5 (C-10b-OAc; C-1a-OAc), and 20.3 (C-2'-OAc).

Mixture **6 and 6'** (30 mg) was acetylated with Ac_2O–pyridine and the reaction mixture was purified over a silica gel column, eluting with a Hex-EtOAc (20%) mixture to give only the compound **6a** (Waxy solid, 10 mg). IR (NaCl) ν_{max} (cm^{-1}): 3500; 1754; 1640; 1602. HR-TOFESIMS *m/z*: 309.0943 $[M+Na]^+$ (calcd. for $C_{13}H_{18}O_7Na$, 309.0950). ^1H NMR ($CDCl_3$, 500 MHz) δ_H: 7.38 (1H, s, H-3), 6.15 (1H, d, 7.4, H-1), 4.13 (1H, m, H-6), 3.74 (3H, s, C-11-OCH_3), 3.09 (1H, m, H-5), 2.66 (1H, t, 7.4, H-9), 2.10 (3H, s, C-1-OAc) 2.04 (1H, m, H-7β), 1.96 (1H, m, H-7α), 1.31 (3H, s, H-10). ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C: 169.2 (C-1-OAc), 168.1 (C-11), 151.2 (C-3), 109.8 (C-4), 89.5 (C-1), 78.8 (C-6), 77.0 (C-8), 50.7 (C-11-OMe), 50.1 (C-9), 47.1 (C-7), 41.3 (C-5), 23.8 (C-1-OAc), 20.9 (C-10),

Mixture **7** and **7'** (120 mg) was acetylated with Ac_2O–pyridine and the reaction mixture was purified over a silica gel column, eluting with a Hex-EtOAc (80%) mixture to give compounds **7a** (20 mg) and **7b** (30 mg).

Compound **7a** was obtained as a waxy solid. IR (NaCl) ν_{max} (cm^{-1}): 3500; 1754; 1640; 1602. HR-TOFESIMS *m/z*: 293.1006 $[M+Na]^+$ (calcd. for $C_{13}H_{18}O_6Na$, 293.1001). ^1H NMR

($CDCl_3$, 600 MHz) δ_H: 7.35 (1H, s, H-3), 6.15 (1H, d, 7.3, H-1), 3.74 (3H, s, C-11-OCH_3), 3.21 (1H, m, H-5), 2.35 (1H, m, H-6), 2.29 (1H, t, 8.5, H-9), 2.10 (3H, s, C-1-OAc), 1.73 (2H, m, H-7), 1.52 (1H, m, H-6), 1.32 (3H, s, H-10). ^{13}C NMR ($CDCl_3$, 150 MHz) δ_C: 169.5 (C-1-OAc), 167.2 (C-11), 150.2 (C-3), 112.0 (C-4), 89.8 (C-1), 79.6 (C-8), 51.3 (C-9), 50.1 (C-11-OMe), 40.3 (C-7), 30.5 (C-5), 29.5 (C-6), 24.3 (C-10), 21.5 (C-1-OAc).

Compound **7b** was obtained as a waxy solid. ^{1}H and ^{13}C NMR spectra showed similar chemical shifts to those of compound **7a** except for the additional peaks of an acetyl group, due to acetylation of the tertiary hydroxyl group at C-8. IR (NaCl) ν_{max} (cm^{-1}): 1754; 1640; 1602. HR-TOFESIMS *m/z*: 335.1111 $[M+Na]^+$ (calcd. for $C_{15}H_{20}O_7Na$, 335.1107]. ^{1}H NMR ($CDCl_3$, 600 MHz) δ_H: 7.41 (1H, s, H-3), 6.15 (1H, d, 7.5, H-1), 3.75 (3H, s, C-11-OMe), 3.16 (1H, m, H-5), 2.68 (1H, t, 7.5, H-9), 2.30 (1H, m, H-6), 2.10 (3H, s, C-1-OAc), 2.05 (1H, m, H-7), 2.04 (3H, m, C-8-OAc) 1.85 (1H, m, H-7), 1.55 (3H, s, H-10), 1.39 (1H, m, H-6). ^{13}C NMR ($CDCl_3$, 150 MHz) δ_C: 170.7 (C-8-OAc), 169.2 (C-1-OAc), 166.9 (C-11), 151.2 (C-3), 111.0 (C-4), 89.5 (C-1), 88.6 (C-8), 51.3 (C-11-OMe), 48.7 (C-9), 38.5 (C-7), 30.9 (C-5), 28.8 (C-6), 22.2 (C-8-OAc), 21.0 (C-1-OAc), 20.5 (C-10).

3.7. Acid Hydrolysis

15 mg of each compound (**2-4**) was individually refluxed in 2N HCl (5.0 mL) at 80 °C for 3h. Each reaction mixture was extracted with $CHCl_3$ (3 × 5mL), and the H_2O phase was dried using a N_2 stream (Gournelis et al., 1989). The residues were separately subjected to CC over silica gel with MeCN/H_2O (8:1) as the eluent to yield glucose (2 mg, 3.3 mg and 3.5 mg respectively), $[\alpha]^{20}_D$ +35.9 (*c* 1.0, H_2O). The solvent system MeCN/H_2O (6:1) was used for TLC identification of glucose (Zhang et al., 2008).

Acknowledgments

The authors thank the University of Dschang, the "Région Champagne-Ardenne and the Département de la Marne" for financial support. The EU-programme FEDER to the PIANET CPER project is also gratefully acknowledged.

References

Achenbach, H. 1986. Investigations on West African medicinal plants. *Pure and Apllied Chemistry* 58, 653-662

Achenbach, H., Waibel R., Addae-mensah I., 1980. Shanzhisin methyl ester gentiobioside, a new iridoid-isolation and synthesis. *Tetrahedron Letters* 21, 3677-3678

Achenbach, H., Waibel R., Addae-mensah I., 1981. Iridoid and other constituents of *Canthium subcordatum*. *Phytochemistry* 20, 1591-1595.

Ampofo, O., 1977. Paper read at the Third Symposium of Medicinal Plants, Ife, Nigeria.

Awah F.M., Uzoegwu, P.N., Ifeonu, P., Oyugi, J. O., Rutherford, J., Yao, X.J., Fehrmann, F., Fowke, K.R., Eze, M.O. 2012. Free radical scavenging activity, phenolic contents and cytotoxicity of selected Nigerian medicinal plants. *Food Chemistry* 13, 1279-1286.

Bailleul, F., Delaveau, P., Rbaron, A., Plat, M., Koch, M., 1977. Feretoside and gardenoside from *Feretia apodanthera*, ^{13}C-NMR spectra iridoïd series. *Phytochemistry* 16, 723-726.

Bickoff, E.M., Witt, S.C., Livingston, A.L., 1965. 3',4',7-hydroxyflavone in *Alfalfa. Journal of Pharmaceutical Sciences*. 54, 1555.

Cui-Lian, F., Ming-Fu, G., Yan-Bo, Z., Hao-Fu, D., Wen-Li, M., 2010. Scyphiphinc, a new Iridoid from *Scyphiphora hydrophyllacea*. *Molecules* 15, 2473-2477.

Demirezer, O.L., Gürbüz, F., Güvernalp, Z., Ströch, K., Zeeck,A., 2006. Iridoïds, flavonoids and monoterpene glycosides, from *Galium verum* subsp. Verum. *Turk. Journal of Chemistry* 30, 525-534.

Dongo E., Ayafor, J.F., Sondengam B.L., Connoly J.D., 1989 A new peptide alkaloïd from *Canthium arnoldianum*. *Journal of Natural Products* 52, 840-843.

Gournelis, D., Skaltsounis, A.L, Tillequin, F., Koch, M., 1989. Plantes de Nouvelle-Calédonie, CXXI. Iridoïdes et alcaloïdes de *Plectronia odorata*. *Journal of Natural Products* 52, 306-316.

Guo, S.J., Gao, L.M., Cheng, D.L., 2001. Iridoid from *Phlomis umbrosa. Pharmazie* 98, 178-180.

Hamerski, L., Furlan, M., Siqueira, D.H., Cavalheiro, A.J., Nogueira, M., Tomazela, D.M., Bolzani, V.S., 2003. Iridoïd glucosides from *Randia spinosa* (Rubiaceae). *Phytochemistry* 63, 397-400.

Hossain, A.M., Ismail, Z., 2013. Isolation and characterization of triterpenes from the leaves of *Orthosiphon stamineus. Arabian Journal of Chemistry* 6, 295-298.

Inouye, H., Takeda, Y., Nishimura, H., 1974. Two new iridoïd glucosides from *Gardenia jasminoides* fruits. *Phytochemistry* 13, 2219-2224.

Irvine, F. R., 1961. Woody Plants of Ghana. Oxford University Press, London, p 658.

Kanchanpoom, T., Kasai, R., Chumsri, P., Yamasaki, K., 2002. Iridoïd glucosides from *Thumbergia laurifolia*. *Phytochemistry* 60, 769-771.

Khatri, L.M., Kazi, M.A., 1979. Chemical investigation of *Aticharis linearis* Hochst, Part- II (structure of linearin and linearoside). *Journal of Chemical Society of Pakistan*. 1, 25-28.

Patro, S.K., Sasmal, D., Mazumndar, P., Behera, P., Lal, U.R., Dash, S.K., Padhy, R.K., 2014. Review on genus Canthium: Special reference to *Canthium coromandelicum*-an unexplored traditional medicinal plant of Indian Subcontinent. *American Journal of Phytomedicine and Clinical Therapeuties* 2, 796-813.

Sholichin M., Yamasaki, K., Kasai, R., Tanaka, O., 1980. ^{13}C-Nuclear magnetic resonance of lupane type triterpenes. Lupeol, betulin and betulinic acid. *Chemical and Pharmaceutical Bulletin.* 28, 1006-1008.

Sui-Kiong, L., Akiko, K., Takashi, T., Toshihiro, F., Kunihide, M., Asao, K., 2002. Iridoïds and anthraquinones from the Malaysian Medicinal Plant, *Saprosmas cortechnii* (Rubiaceae). *Chemical and Pharmaceutical Bulletin*. 50, 1035-1040.

Takeda, Y., Nishimura, H., Inouye, H., 1997. Two new iridoid glucosides from *Mussaenda parviflora* and *Mussaenda shikokinia*. *Phytochemistry* 16, 1401-1404.

Yahara, S., Kato, K., Nakazawa, Y., Toda, Y. Nohara, T., 1990. New iridoïds trimmers and tetramers from seeds of *Eucommia ulmoides*. *Chemical and Pharmaceutical Bulletin.* 38, 267-269.

Zapesochnaya, G.G., Kurkin, V.A., Pervykh, I.N., Karasartov, B.S., 1991. Velpetin-a new iridoid glycoside from *Nepeta velutina*. *Khimija Prirodnykh Soedineny* 6, 777-781.

Zeng, Y.B., Mei, W.L., Wang, H., Li, X.N., Dai, H.F., 2010. Scyphiphin D, a new iridoid glucoside dimer from *Scyphiphora hydrophyllacea. Journal of Asian Natural Products Research* 12, 1010-1014.

Zhang, F., Sun, L., Chen, W.S., 2009. A new iridoid glucoside from *Lamiophlomis rotata*. *Chemistry of Natural Compounds* 45, 360-362.

Zhang, Y., Gan, M., Lin, S., Liu, M., Song, W., Zi, J., Wang, S. Li, S., Yang, Y., Shi, J., 2008. Glycosides iridoïd from the bark of *Adina polycephala*. *Journal of Natural Products* 71, 905-909.

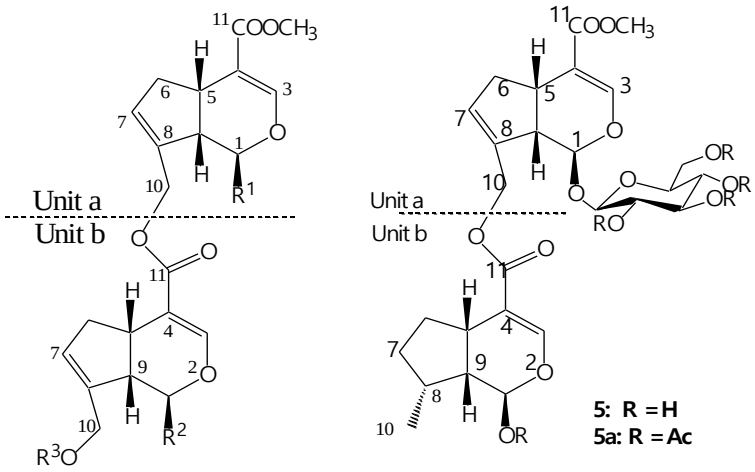
Table 1: ^{1}H-NMR Spectral data of Compounds **1-4** (CD_3OD) and **5a** ($CDCl_3$)

Positions	Compounds				
	1 (500 MHz)	**2** (600 MHz)	**3** (500 MHz)	**4** (500 MHz)	**5a** (600 MHz)
1a	4.84 (1H, d, 8.6)	4.84 (1H, d, 8.6)	5.24 (1H, d, 7.5)	5.24 (1H, d, 7.5)	5.22 (1H, d, 7.8)
3a	7.55 (1H, s)	7.55 (1H, d, 0.7)	7.54 (1H, d, 0.9)	7.54 (1H, d, 1.1)	7.43 (1H, s)
4a	-	-	-	-	-
5a	3.18 (1H, q, 8.5)	3.18 (1H, m)	3.23 (1H, m)	3.23 (1H, m)	3.22 (1H, m)
6a	2.87 (1H, m, H-β) 2.06 (1H, m, H-α)	2.87 (1H, m, H-β) 2.08 (1H, m, H-α)	2.15 (1H, ddt, 16.4, 7.8, 2.3, H-α) 2.87 (1H, dd, 16.4, 7.8, H-β)	2.13 (1H, ddt, 16.4, 7.7, 2.3, H-α) 2.87 (1H, dd, 16.4, 7.7, H-β)	2.88 (1H, m, H-β) 2.21 (1H, m, H-α)
7a	5.91 (1H, br s)	5.91 (1H, br s)	5.87 (1H, brs)	5.83 (1H, brs)	5.89 (1H, br, s)
8a	-	-	-	-	-
9a	2.56 (1H, t, 8.5)	2.57 (1H, t, 7.8)	2.81 (1H, t, 7.7)	2.81 (1H, t, 7.7)	2.94 (1H, t, 7.8)
10a	4.91 (1H, d, 14.4) 4.78 (1H, d, 14.4)	4.91 (1H, d, 14.2) 4.82 (1H, d, 14.2)	4.82 (1H, d, 13.9) 4.94 (1H, d, 13.9)	4.81 (1H, dd, 14.1, 1.4) 4.95 (1H, d, 14.1)	4.85 (1H, d, 13.8) 4.72 (1H, d, 13.8)
11a	-	-	-	-	-
11a-OMe	3.73 (3H, *s*)	3.72 (3H, *s*)	3.74 (3H, s)	3.74 (3H, s)	3.75 (3H, s)
1'a	-	-	4.73 (1H, d, 7.8)	4.75 (1H, d, 7.9)	4.90 (1H, d, 7.9)
2'a	-	-	3.25 (1H, dd, 9.2, 7.8)	3.25 (1H, dd, 9.2, 7.9)	5.05 (1H, dd, 8.6, 7.9)
3'a	-	-	3.40 (1H, t, 8.8)	3.40 (1H, t, 9.1)	5.25 (1H, t, 8.6)
4'a	-	-	3.31 (1H, m)	3.32 (1H, m)	5.13 (1H, dd, 9.5, 8.6)
5'a	-	-	3.31 (1H, m)	3.32 (1H, m)	3.75 (1H, m)
6'a	-	-	3.67 (1H, dd, 12.0, 5.5) 3.89 (1H, d, 12.0)	3.67 (1H, dd, 11.8, 5.6) 3.89 (1H, d, 11.8)	4.15 (1H, dd, 12.2, 5.8) 4.28 (1H, dd, 12.2, 2.3)
1b	4.82 (1H, d, 8.6)	5.22 (1H, d, 7.7)	5.21 (1H, d, 7.7)	5.21 (1H, d, 7.6)	6.05 (1H, d, 7.7)
3b	7.61 (1H, s)	7.60 (1H, s)	7.61 (1H, d, 0.8)	7.60 (1H, d, 1.1)	7.43 (1H, s)
4b	-	-	-	-	-
5b	3.21 (1H, q, 8.5)	3.25 (1H, m)	3.24 (1H, m)	3.23 (1H, m)	2.98 (1H, m)
6b	2.06 (1H, m, H-α) 2.87 (1H, m, H-β)	2.17 (1H, m, H-α) 2.88 (1H, m, H-β)	2.18 (1H, ddt, 16.4, 8.1, 2.1, H-α) 2.90 (1H, dd, 16.4, 8.1, H-β)	2.13 (1H, ddt, 16.4, 7.7, 2.3, H-α) 2.86 (1H, dd, 16.4, 7.7, H-β)	2.18 (1H, m, H-β) 1.59 (1H, m, H-α)
7b	5.91 (1H, br s)	5.88 (1H, br s)	5.89 (1H, brs)	5.83 (1H, brs)	1.87 (1H, m, H-β) 1.39 (1H, m, H-α)
8b	-		-	-	2.35 (1H, m)
9b	2.56 (1H, t, 8.5)	2.80 (1H, t, 7.8)	2.80 (1H, t, 7.0)	2.77 (1H, t, 7.9)	2.25 (1H, m)
10b	4.81 (1H, d, 14.5) 4.76 (1H, d, 14.5)	4.80 (2H, brs)	4.81 (2H, m)	4.21 (1H, dd, 14.3, 1.5) 4.34 (1H, d, 14.3)	1.05 (3H, d, 5.1)
10b-OAc	2.21 (3H, *s*)	2.10 (3H, s)	2.09 (3H, s)	-	-
11b	-	-	-		-
1'b	-	4.74 (1H, d, 7.8)	4.74 (1H, d, 7.9)	4.73 (1H, d, 7.8)	-
2'b	-	3.24 (1H, dd, 9.1, 7.8)	3.24 (1H, dd, 8.7, 7.8)	3.25 (1H, dd, 9.0, 7.8)	-
3'b	-	3.40 (1H, t, 9.1)	3.39 (1H, t, 8.9)	3.41 (1H, t, 9.0)	-
4'b	-	3.31 (1H, m)	3.31 (1H, m)	3.32 (1H, m)	-
5'b	-	3.31 (1H, m)	3.31 (1H, m)	3.32 (1H, m)	-
6'b	-	3.67 (1H, dd, 11.7, 5.8) 3.89 (1H, dd, 11.7, 1.7)	3.67 (1H, m) 3.89 (1H, d, 12.1)	3.68 (1H, dd, 12.0, 5.6) 3.89 (1H, d, 12.0)	-
1b-OAC ($GlcAc_4$)		-	-	-	2.15 (3H, s) 2.11, 2.07, 2.03, 1.98

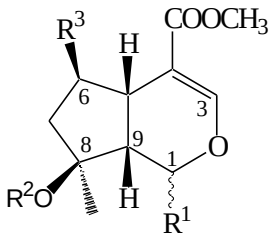
Table 2: ^{13}C-NMR Spectral data of Compounds **1-4** (CD_3OD) and **5a** ($CDCl_3$)

Positions	**Compounds**				
	1 (125 MHz)	**2** (150 MHz)	**3** (125 MHz)	**4** (125 MHz)	**5a** (150 MHz)
1a	96.2	96.1	96.8	96.8	95.5
3a	152.9	152.9	151.9	152.0	150.9
4a	110.2	110.1	111.0	111.0	112.1
5a	35.9	35.9	34.9	34.9	33.5
6a	38.8	38.7	38.6	38.5	38.4
7a	129.3	129.4	129.7	127.0	130.8
8a	139.3	139.3	138.1	138.3	136.9
9a	47.2	47.2	46.3	46.3	46.8
10a	62.1	62.1	61.9	61.9	61.4
11a	168.4	168.0	168.0	168.1	167.3
11a-OMe	50.3	50.3	50.4	50.4	51.3
1’a	-	-	98.9	98.9	96.1
2’a	-	-	73.4	73.4	70.7
2a’-OAc					20.3 and 169.1
3’a	-	-	76.4	76.4	72.5
3’a-OAc					20.6 and 170.2
4’a	-	-	70.1	70.0	68.1
4’a-OAc					20.7 and 169.4
5’a	-	-	77.0	77.0	72.1
6’a	-	-	61.3	61.3	61.7
6’a-OAc					20.7 and 170.6
1b	96.3	96.8	96.8	96.9	90.7
3b	153.2	152.2	152.2	152.3	151.1
4b	110.2	111.0	111.0	111.2	111.8
5b	36.0	35.0	35.0	35.2	33.7
6b	38.7	38.6	38.2	38.4	31.2
7b	129.7	129.9	130.0	129.7	31.8
8b	139.1	138.4	138.3	143.3	36.2
9b	47.0	46.0	45.9	45.7	42.0
10b	62.5	62.3	62.3	60.0	16.0
11b	167.6	167.3	167.4	167.5	166.4
10b-OAc	171.3	171.4	171.4	-	-
10b-OAc	19.4	19.4	19.4	-	-
1’b	-	99.0	99.2	99.2	-
2’b	-	73.4	73.5	73.5	-
3’b	-	76.5	76.5	76.5	-
4’b	-	70.1	70.1	701	-

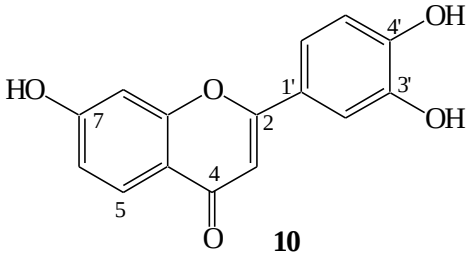
5’b	-	77.0	77.0	77.0	-
6’b	-	61.4	61.4	61.4	-



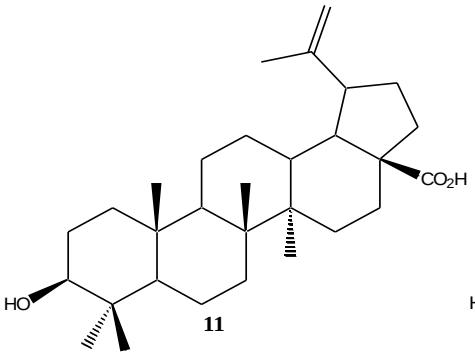
	R^1	R^2	R^3
1	OH	OH	Ac
2	OH	*O*-Glc	Ac
2a	OAc	*O*-GlcAc$_4$	Ac
3	*O*-Glc	*O*-Glc	Ac
4	*O*-Glc	*O*-Glc	H



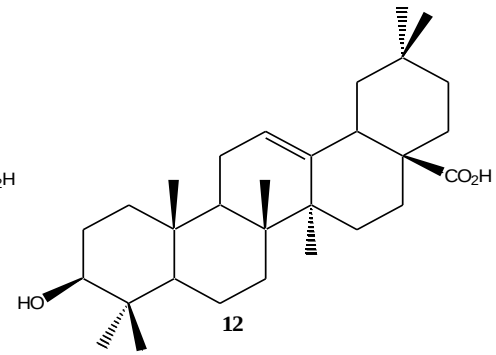
	R^1	R^2	R^3
6	β-OH	OH	H
6'	α-OH	OH	H
6a	β-OAc	OH	H
7	β-OH	H	H
7'	α-OH	H	H
7a	β-OAc	H	H
7b	β-OAc	H	Ac
8	β-*O*-Glc	H	H
9	β-*O*-Glc	OH	H



10



11



12

Fig. 1. Chemical structure of the isolated compounds (1-12).