Triterpenoid Derivatives from *Cylicodiscus gabunensis*

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Three new olean-12-ene derivatives (1—3), together with known urs-12-ene-3β, 28-diol (4) were isolated from the stem root of *Cylicodiscus gabunensis*. The structures of the new compounds were established by chemical and spectroscopic means as β-amyrin-n-nonyl ether (1), 22α-hydroxyolean-12-en-3β-yl-β-D-galactopyranoside (2), and 24-hydroxyolean-12-en-3β-yl-β-D-glucopyranoside (3).

**Key words** *Cylicodiscus gabunensis*; Mimosaceae; olean-12-ene; triterpene; β-amyrin derivative; triterpene glycoside

The *Cylicodiscus* genus belongs to the Mimosaceae family comprising 64 genera and 2950 species, mostly tropical. 1) *Cylicodiscus gabunensis* Harm (C. gabunensis), known as Denya (Ghana), Edum (Gabon), Adoum, Bokoka (Cameroon), or Bouemon (Ivory Coast), is a large tree, common in the rain forests of Sierra Leone to the Cameroon and Gabon. 2—4) In the traditional medicine its stem bark is used for various therapeutic purposes. 5—7) Preliminary bioassays on the crude EtOAc extract from the stem bark of *C. gabunensis* have shown antidiarrheal and antimicrobial activities. 6) These results may be explained by the presence of triterpenoids, a class of phytochemical compounds known for their broad spectrum of biological properties. 7—10) Recently, we reported cal investigation of *C. gabunensis* contained coumestan and known urs-12-ene-3β, 28-diol (4) were isolated as white powder and gave a positive Lieberman–Burchard test for saponin. The 1H-NMR spectrum of compound 2 showed a tertiary methyl group (δH 0.76, 0.86, 0.88, 0.92, 0.95, 0.98, 1.03, 1.08 (each 3H, s)], an olefinic proton (δH 5.23 (1H, t, J = 3.6 Hz)], and two oxygenated CH groups (δH 3.35 (1H, dd, J = 3.6, 11.1 Hz), 3.40 (1H, dd, J = 13.7, 4.3 Hz)]. Moreover, the 13C-NMR and 13C-DEPT spectra of aglycone indicated characteristic signals of two oxygenated C-atoms (δC 81.4, 76.6 (each CH2), and two.

Results and Discussion

The crude methanolic extract of the stem root of *C. gabunensis* was successively extracted with hexane, and EtOAc to give 15.8 g and 122 g of extract, respectively. A portion of the EtOAc extract was subjected to column chromatography (CC) as described in the Experimental to afford compounds 1, 2, 3, and 4.

Compound 1 was isolated as white powder and gave a positive Lieberman–Burchard test for triterpenoids. Combined with broad-band-decoupled 13C-NMR and distortionless enhancement by polarization transfer (DEPT) analysis, its molecular formula was determined as C63H106O by high resolution-electron ionization-MS (HR-EI-MS) spectrum, showing a molecular ion peak (M+) at m/z = 604.4338 consistent with the molecular formula C63H106O. The positive response to the Lieberman–Burchard test, as well as inspection of the 13C- and 1H-NMR spectral data revealed 2 to be a saponin. The 1H-NMR spectrum of aglycone revealed eight tertiary methyl groups [δH 0.76, 0.86, 0.88, 0.92, 0.95, 0.98, 1.03, 1.08 (each 3H, s)], an olefinic proton [δH 5.23 (1H, t, J = 3.6 Hz)], and two oxygenated CH groups [δH 3.35 (1H, dd, J = 3.6, 11.1 Hz), 3.40 (1H, dd, J = 13.7, 4.3 Hz)]. Moreover, the 13C-NMR and 13C-DEPT spectra of aglycone indicated characteristic signals of two oxygenated C-atoms [δC 81.4, 76.6 (each CH2), and two.

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olefinic C-atoms [$\delta_c$ 122.5 (CH), 143.9 (C)]. Comparison of the $^1$H- and $^{13}$C-NMR assignments of 2, which were established by analysis of the $^1$H–$^1$H COSY, HMOC, and HMBC spectra, with reported data suggested that the aglycone moiety was olean-12-ene-3β,22-diol (α-soporadiol).

Table 1. The $^1$H-NMR ($\delta_h$ in ppm, 500 MHz) and $^{13}$C-NMR ($\delta_c$ in ppm, 125 MHz) Data of Compounds 1–3

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>$\delta_h$ (mult., $J_{hh}$)</th>
<th>$\delta_c$ (mult., $J_{cc}$)</th>
<th>$\delta_h$ (DEPT)</th>
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a) Measured in CDCl$_3$; b) Measured in DMSO-$d_6$.

Meanwhile, the configuration of the β-galactopyranosyl unit was deduced to be β from the coupling constant value observed for the H-1′ of Gal ($J$ = 7.8 Hz). HMBC correlations from H-1′ (δH 4.80) of galactose unit to C-3 (δC 81.4) of the aglycone confirmed the attachment of the β-galactopyranosyl group to C-3. On the basis of the above data, the structure of compound 2 was elucidated to be 22α-hydroxyolean-12-en-3β,22-diol-β-D-galactopyranoside (2) (see Fig. 1).

Compound 3 was obtained as colourless needles. Its molecular formula was deduced as C$_{36}$H$_{60}$O$_7$ by HR-FAB-MS.
[m/z=604.4338 (M+)] and confirmed by broad band decoupled $^{13}$C-NMR and $^{1}$H-DEPT analysis. The olean-12-ene glycoside nature of 3 was evident from the positive response to the Lieberman–Burchard test combined with the $^{13}$C- and $^{1}$H-NMR spectral data analysis. Comparison of the $^{1}$H- and $^{13}$C-NMR assignments of 3, which were established under the same conditions as in the case of 1 and 2, with reported data suggested that aglycone moiety and carbohydrate unit were olean-12-ene-3$\beta$, 24-diol[14] and $\beta$-d-glucose,[15,16] respectively. Acid hydrolysis followed by spectroscopic analysis of the aglycone and direct HPLC analysis of the sugar component, confirmed this suggestion. Furthermore, the configuration of the $\beta$-d-glucopyranosyl moiety was regarded to be $\beta$ by the $J$ value of its anomeric proton signal at $\delta$ 5.32 (d, J = 7.9 Hz). The site of glycosylation was suggested by a downfield shift observed for C-3 ($\delta$ 81.9 in 3, this signal appeared at 80.7 ppm in olean-12-ene-3$\beta$, 24-diol), and confirmed by HMBC experiments showing correlations between H-1 (d$\beta$ 5.32) of glucosidic unit and C-3 ($\delta$ 81.9) of the aglycone. Consequently, the structure of 3 was determined as 24-hydroxylean-12-en-3$\beta$-yl-$\beta$-d-glucopyranoside (3) (see Fig. 1). The known compound urs-12-ene-3$\beta$, 28-diol (4) was isolated as white powder and identified by comparison with the reported data.[12]

Experimental

General Procedures

Melting points were determined on X-4 digital micro-melting point apparatus and were uncorrected. Optical rotations were measured with a Perkin-Elmer 341 digital polarimeter. IR spectra were recorded with KBr pellets on a Perkin-Elmer 577 spectrometer. HPLC was performed by using a system comprised of a CPCM pump, a CCP PX-8010 controller, an RI-8010 detector and a Shodex OR-2 detector, and a Rheodyne injection port with a 20 μl sample loop. The EI-MS was recorded on a JEOL JMS-300 mass spectrometer. The FAB-MS was obtained with a Kratos MS 25 instrument with a DS-55 data system, and collision gas Xe (ion gun 22 mm, 0.25 mtorr). The retention times and optical rotation with those of an authentic sample: retention time and optical rotation with those of an authentic sample: 15)

Plant Material

The stem root of Cylindrocyclus gabunensis HAMS was collected in May 2002 on Mount Eloudem, Yaounde-Cameroon. The plant was identified at the National Herbarium, Yaounde and identified, where a voucher specimen is deposited (No. 21574/SRF/CAM).

Extraction and Isolation

The air-dried, powdered stem root of C. gabunensis (4.8 kg) was immersed in MeOH (25 l) and kept for 72 h. The MeOH extract was filtered and concentrated to dryness under reduced pressure. The crude extract (225 g) was successively extracted with n-hexane, (5×500 ml) and EtOAc (5×500 ml) to give 15.8 g and 122 g of extract, respectively and 67.5 g of residue. A 90 g portion of the EtOAc extract was subjected to CC on silica gel (400 g) using a gradient solvent system of hexane–EtOAc, EtOAc–MeOH and MeOH in increasing polarity. A total of 200 fractions of 250 ml each were collected and combined on the basis of TLC analysis leading to six main series I—VI. Series III (9.5 g) [fractions 20—49] was rechromatographed on silica gel, using hexane–EtOAc (70:30) to give fifty fractions (F1—F50). Fractions F3 and F7 afforded compound 1 (200 mg) and 4 (150 mg) respectively. Series V (3 g) [fractions 150—190] was separated on Sephadex LH-20 eluted with MeOH to give twenty fractions [F1—F20]. Fraction F18 was further purified by preparative TLC, using MeOH/CH$_2$Cl$_2$/cyclohexane (1.5:5:3.5) to give compounds 2 (32 mg) and 3 (28 mg).

Acknowledgment

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References