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Calotroposide S, New Oxypregnane Oligoglycoside from *Calotropis procera* Root Bark

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Abstract: Calotroposide S (**1**), a new oxypregnane oligoglycoside has been isolated from the *n*-butanol fraction of *Calotropis procera* (Ait) R. Br. root bark. The structure of **1** was assigned based on various spectroscopic analyses. Calotroposide S (**1**) possesses the 12-O-benzoylisolineolon aglycone moiety with eight sugar residues attached to C-3 of the aglycone. It showed potent anti-proliferative activity towards PC-3 prostate cancer, A549 non-small cell lung cancer (NSCLC), and U373 glioblastoma (GBM) cell lines with IC_{50} 0.18, 0.2, and 0.06 µM, respectively compared with cisplatin and carboplatin.

Keywords: *Calotropis procera*; Asclepiadaceae; calotroposide S; anti-proliferative activity.

1. Plant Source

In the course of continuous work on *Calotropis procera*, a new oxypregnane oligoglycoside named calotroposide S (**1**) was isolated from *n*-BuOH fraction (Fig. 1). Herein, the isolation and structural determination as well as the anti-proliferative activity of **1** towards different cancer cell lines are discussed. The root barks of *Calotropis procera* were collected from Ismailia in April 2009. Identification of the plant was done by Prof. Dr. A. Fayed (Faculty of Science, Assiut University, Assiut, Egypt). A voucher specimen under registration code DY-CP-2009 was kept at the Pharmacognosy Department Herbarium, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt.

2. Previous Studies

Calotropis procera belonging to Asclepiadaceae family is a wild-growing plant. It possesses different biological activities: antitumor, analgesic, anti-inflammatory, anti-diarrheal, antioxidant, hepatoprotective, antiulcer, insecticidal, anthelmintic, antibacterial, and spasmolytic [1-7]. Previously, we have reported on the isolation of cardiac glycoside, oxypregnane oligoglycosides, and ursane-type triterpenes from *Calotropis procera* root bark [1-3].

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3. Present Study

The powdered root bark (1.2 kg) was extracted with MeOH (4 × 3.5 L). The obtained extracts were concentrated to give a brownish residue (55.0 g). The latter was mixed with 400 mL distilled water, followed by successive extraction with *n*-hexane (4 times each 400 mL), $CHCl_3$ (4 times each 400 mL), EtOAc (4 times each 400 mL), and *n*-butanol (3 times each 400 mL) to give 4.5, 3.2, 5.6, and 7.5 g, respectively. VLC of the anticancer *n*-BuOH fraction using $CHCl_3$/MeOH gradient afforded subfractions: A (1.6 g), B (1.9 g), C (1.8 g), and D (2.1 g). Fractions A-C were investigated previously [3]. SiO_2 column of fraction D (2.1 g) using $CHCl_3$/MeOH gave subfractions D-4A (940 mg) and D-4B (560 mg). Subfraction D-4A was subjected to Sephadex LH-20, then SiO_2 column with $CHCl_3$/MeOH to afford impure **1**, which further purified on HPLC (YMC-ODS-AQ, 250 x 20 mm) using CH_3CN/H_2O (25:75→50:50) to afford **1** (21.4 mg, yellow oil).

*Calotroposide S (**1**):* Yellow oil; $[\alpha]_D$ + 9.4 (*c* 1.1, MeOH); UV (MeOH) λ_{max} (log ε): 228 (4.09), 275 (3.10), 280 (3.12) nm; IR (KBr) ν_{max}: 3510, 2960, 1715, 1623, 1480, 1055 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): **Agly**; δ_H 1.68 (1H, m, H-1A), 1.14 (1H, m, H-1B), 1.69 (1H, m, H-2A), 1.25 (1H, m, H-2B), 3.81 (1H, m, H-3), 2.11 (1H, m, H-4A), 1.73 (1H, m, H-4B), 5.38 (1H, brs, H-6), 2.05 (1H, m, H-7), 1.52 (1H, dd, *J* = 12.8, 3.2 Hz, H-9), 1.85 (1H, m, H-11A), 1.62 (1H, m, H-11B), 4.95 (dd, *J* = 12.0, 3.8 Hz, H-12), 1.94 (1H, m, H-15A), 1.65 (1H, m, H-15B), 2.00 (1H, m, H-16A), 1.61 m (1H, m, H-16B), 3.21 (1H, dd, *J* = 9.5, 5.4 Hz, H-17), 1.65 (3H, s, H-18), 1.13 (3H, s, H-19), 2.03 (3H, s, H-21), 7.97 (2H, dd, *J* = 7.6, 1.8 Hz, H-2`, 6`), 7.44 (2H, t, *J* = 7.6 Hz, H-3`, 5`), 7.55 (1H, dt, *J* = 7.6, 1.8 Hz, H-4`); **Cym-1**: 4.86 (1H, dd, *J* = 9.5, 2.5 Hz, H-1), 1.92 (1H, m, H-2A), 1.46 (1H, m, H-2B), 3.65 (1H, m, H-3), 3.22 (1H, m, H-4), 3.78 (1H, m, H-5), 1.21 (3H, d, *J* = 6.0 Hz, H-6), 3.49 (3H, s, 3-OCH_3); **Cym-2**: 4.83 (1H, dd, *J* = 9.5, 2.4 Hz, H-1), 1.91(1H, m, H-2A), 1.45 (1H, m, H-2B), 3.62 (1H, m, H-3), 3.24 (1H, m, H-4), 3.80 (1H, m, H-5), 1.22 d (3H, d, *J* = 6.5 Hz, H-6), 3.42 (3H, s, 3-OCH_3); **Ole-3**: 4.74 (1H, dd, *J* = 10.0, 2.0 Hz, H-1), 2.10 (1H, m, H-2A), 1.71 (1H, m, H-2B), 3.14 (1H, m, H-3), 3.18 (1H, m, H-4), 3.28 (1H, m, H-5), 1.23 (3H, d, *J* = 6.2 Hz, H-6), 3.49 (3H, s, 3-OCH_3); **Ole-4**: 4.73 (1H, dd, *J* = 10.2, 2.0 Hz, H-1), 2.13 (1H, m, H-2A), 1.74 (1H, m, H-2B), 3.13 (1H, m, H-3), 3.22 (1H, m, H-4), 3.16 (1H, m, H-5), 1.27 (3H, d, *J* = 6.1 Hz, H-6), 3.39 (3H, s, 3-OCH_3); **Cym-5**: 4.69 (1H, dd, *J* = 9.5, 2.2 Hz, H-1), 1.90 (1H, m, H-2A), 1.48 (1H, m, H-2B), 3.64 (1H, m, H-3), 3.19 (1H, m, H-4), 3.82 (1H, m, H-5), 1.29 (3H, d, *J* = 6.3 Hz, H-6), 3.41 (3H, s, 3-OCH_3); **Ole-6**: 4.62 (1H, dd, *J* = 10.1, 1.0 Hz, H-1), 2.15 (1H, m, H-2A), 1.72 (1H, m, H-2B), 3.11 (1H, m, H-3), 3.17 (1H, m, H-4), 3.25 (1H, m, H-5), 1.31 (3H, d, *J* = 6.4 Hz, H-6), 3.41 (3H, s, 3-OCH_3); **Ole-7**: 4.53 (1H, dd, *J* = 10.8, 2.5 Hz, H-1), 2.16 (1H, m, H-2A), 1.75 (1H, m, H-2B), 3.14 (1H, m, H-3), 3.20 (1H, m, H-4), 3.24 (1H, m, H-5), 1.34 (3H, d, *J* = 6.2 Hz, H-6), 3.40 (3H, s, 3-OCH_3); **Ole-8**: 4.52 (1H, dd, *J* = 10.2, 2.0 Hz, H-1), 2.18 (1H, m, H-2A), 1.76 (1H, m, H-2B), 3.15 (1H, m, H-3), 3.26 (1H, m, H-4), 3.41 (1H, m, H-5), 1.36 d (3H, d, *J* = 6.3 Hz, H-6), 3.40 (3H, s, 3-OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): **Agly**; δ_H 38.7 (C-1), 28.9 (C-2), 74.8 (C-3), 37.3 (C-4), 140.8 (C-5), 117.4 (C-6), 34.3 (C-7), 75.4 (C-8), 43.9 (C-9), 35.8 (C-10), 24.3 (C-11), 73.4 (C-12), 55.4 (C-13), 86.7 (C-14), 33.5 (C-15), 21.6 (C-16), 59.7 (C-17), 15.0 (C-18), 18.8 (C-19), 209.9 (C-20), 32.0 (C-21), 130.2 (C-1`), 129.5 (C-2`, 6`), 128.3 (C-3`, 5`), 132.9 (C-4`), 164.1 (C-7`); **Cym-1**: 95.9 (C-1), 35.2 (C-2), 76.7 (C-3), 81.9 (C-4), 68.2 (C-5), 18.0 (C-6), 58.3 (3-OCH_3); **Cym-2**: 99.2 (C-1), 36.7 (C-2), 77.1 (C-3), 82.2 (C-4), 68.3 (C-5), 17.6 (C-6), 58.0 (3-OCH_3); **Ole-3**: 99.7 (C-1), 37.1 (C-2), 77.4 (C-3), 82.3 (C-4), 70.3 (C-5), 17.8 (C-6), 56.5 (3-OCH_3); **Ole-4**: 100.3 (C-1), 36.8 (C-2), 77.4 (C-3), 82.6 (C-4), 71.4 (C-5), 18.2 (C-6), 56.4 (3-OCH_3); **Cym-5**: 100.4 (C-1), 35.3 (C-2), 76.7 (C-3), 80.7 (C-4), 68.7 (C-5), 18.2 (C-6), 58.2 (3-OCH_3); **Ole-6**: 100.3 (C-1), 36.5 (C-2), 77.4 (C-3), 82.3 (C-4), 71.8 (C-5), 18.3 (C-6), 56.6 (3-OCH_3); **Ole-7**: 101.0 (C-1), 36.2 (C-2), 77.1 (C-3), 80.3 (C-4), 69.8 (C-5), 17.9 (C-6), 56.8 (3-OCH_3); **Ole-8**: 101.4 (C-1), 36.4 (C-2), 82.6 (C-3), 77.8 (C-4), 72.4 (C-5), 18.4 (C-6), 56.7 (3-OCH_3); HRESIMS *m/z* 1619.8729 $[M - H]^+$ (calcd for $C_{84}H_{131}O_{30}$, 1619.8725).

The anti-proliferative activity of **1** was determined against the PC-3 prostate cancer (DSMZ; code ACC465), A549 NSCLC (DSMZ; code ACC107), and U373 GBM (ECACC; code 89081403) cancer cell lines using MTT colorimetric assay as previously outlined [1-3].

Compound **1** was isolated as yellow oil and gave positive Keller-Kiliani and Libermann-Burchard reactions suggesting that **1** had steroidal skeleton containing 2-deoxy sugar [8]. It gave a HRESIMS

pseudo-molecular ion peak at *m/z* 1619.8729 $[M - H]^+$ (calcd for 1619.8725), attributable to a molecular formula of $C_{84}H_{132}O_{30}$. It possesses three degrees of unsaturation and 432 mass units more than calotroposide H, which was previously isolated from *C. procera* [3]. It had IR absorptions at 3510 (OH), 1715 (C=O), 1623, and 1055 cm^{-1}. The UV spectrum revealed absorptions at 228, 275, and 280 nm, indicating the presence of benzoyl moiety. The ^1H and ^{13}C spectra of **1** exhibited signals for a *tri*-substituted olefinic double bond at δ_H 5.38 (brs, H-6)/δ_C 117.4 (C-6) and 140.8 (C-5) and three methyls at δ_H 2.03 (s, H_3-21)/δ_C 32.0 (C-21), 1.65 (s, H_3-18)/δ_C 15.0 (C-18), and 1.13 (s, H_3-19)/δ_C 18.8 (C-19) characteristic for the presence of pregn-5-en-20-one skeleton in **1** [3,9-12]. The location of the olefinic double bond at C-5-C-6 was established based on the $^3J_{CH}$ HMBC cross peaks of H-6 with C-8 (δ_C 75.4) and C-10 (δ_C 35.8) and H-3 and H_3-19 with C-5. The a benzoyl moiety was evident by the signals at δ_H 7.97 (dd, *J* = 7.6, 1.8 Hz, H-2`, 6`)/δ_C 129.5 (C-2`, 6`), 7.44 (t, *J* = 7.6 Hz, H-3`, 5`)/δ_C 128.3 (C-3`, 5`), 7.55 (dt, *J* = 7.6, 1.8 Hz, H-4`)/$\delta_C$ 132.9 (C-4`), 130.2 (C-1`), and 164.1 (C-7`). The observed ^1H-^1H COSY cross peaks of H-3`/H-2` and H-4` and H-5`/H-4` and H-6` confirmed the presence of this moiety. This was further proved by the observed cross peaks of H-2` and H-6`/C-1`, C-4`, and C-7`, H-4`/C-2` and C-6`, and H-3` and H-5`/C-1` and C-4` in the HMBC. The location of this moiety at C-12 was established by the observed HMBC correlation between H-12/C-7`. Furthermore, the ^{13}C NMR spectrum exhibited two oxygen-bonded quaternary carbons at δ_C 75.4 (C-8) and 86.7 (C-14). The cross peaks of H-6/C-8, H-15/C-8, H-17/C-14, and H-18/C-14 confirmed the assignment of these carbons. Moreover, signals for two oxymethine groups were observed at δ_H 3.81 (m, H-3) and 4.95 (dd, *J* = 12.0, 3.8 Hz, H-12). They correlated to the carbon signals resonating at δ_C 74.8 and 73.4, respectively in the HMQC. Their positions at C-3 and C-12 were established by the observed HMBC correlations of H-1 and H-4 to C-3 and H-9 and H_3-18 to C-12. The signals at δ_H 3.21 (dd, *J* = 9.5, 5.4 Hz, H-17)/δ_C 59.7 (C-17) indicated the presence of α-oriented H-17 [3,9-12], which was confirmed by the cross peaks of H-12, H-15, and H-18/C-17 observed in HMBC spectrum. Comparing of the NMR data of **1** with literature supported the assignment of aglycone as 12-*O*-benzoylisolineolon [3,9-12]. Moreover, eight anomeric protons signals at δ_H 4.86 (dd, *J* = 9.5, 2.5 Hz, H-1 of Cym-1), 4.83 (dd, *J* = 9.5, 2.4 Hz, H-1 of Cym-2), 4.74 (dd, *J* = 10.0, 2.0 Hz, H-1 of Ole-3), 4.73 (dd, *J* = 10.2, 2.0 Hz, H-1 of Ole-4), 4.69 (dd, *J* = 9.5, 2.2 Hz, H-1 of Cym-5), 4.62 (dd, *J* = 10.1, 1.0 Hz, H-1 of Ole-6), 4.53 (dd, *J* = 10.8, 2.5 Hz, H-1 of Ole-7), and 4.52 (1H, dd, *J* = 10.2, 2.0 Hz, H-1 of Ole-8) were observed in the ^1H NMR spectrum. They showed HMQC cross peaks to the carbons at δ_C 95.9, 99.2, 99.7, 100.3, 100.4, 100.3, 101.0, and 101.4 respectively, indicating the presence of eight monosaccharide moieties in **1**. The doublet methyl signals at δ_H 1.21, 1.22, 1.23, 1.27, 1.29, 1.31, 1.34, and 1.36 and the methoxy groups at δ_H 3.49 (2 x OCH_3), 3.42, 3.41 (2 x OCH_3), 3.40 (2 x OCH_3), and 3.39 suggested that **1** had eight 3-*O*-methyl deoxy sugar moieties in. The anomeric protons configurations were assigned as *β* based on the $^3J_{H\text{-}1,H\text{-}2(ax)}$ values (9.5-10.8 Hz) [3]. It was suggested that **1** is an octaoside based containing five *β*-D-oleandropyranose and three *β*-D-cymaropyranose units by comparing its NMR spectral data with the previously reported calotroposides H-N [3,13,14]. NMR data of **1** were similar to those of calotroposide H except the presence of three additional oleandrose moieties [3,12]. This was further proved by the observed ESIMS peaks at *m/z* 1474.5 $[(M - H) - 145 (Ole)]^+$, 1225.8 $[(M - H) - 394 (benzoyl group+2 Ole)]^+$, and 1081.2 $[(M - H) - 538 (benzoyl group+3 Ole)]^+$. Their attachment was proved to be 1→4 based on the observed HMBC correlations of δ_H 4.62 (H-1 of Ole-6) with δ_C 80.7 (C-4 of Cym-5), δ_H 4.53 (H-1 of Ole-7) with δ_C 82.3 (C-4 of Ole-6), and δ_H 4.52 (H-1 of Ole-8) with δ_C 80.3 (C-4 of Ole-1).

The identification of sugars in the hydrolysate of **1** was established by co-TLC with authentic sugars as well as comparing the retention times obtained in GCMS with standard monosaccharides). The GCMS chromatogram revealed that the ratio between cymaropyranose and oleandropyranose moieties is 3:5 (See Supporting Information). The deoxy sugars absolute configuration was assessed to be D-form by comparing the optical rotation and ^{13}C data with those of the corresponding sugars [3,11,12,14]. The connectivities of sugars at C-3 was established from HMBC cross peaks between δ_H 4.86 (H-1 of Sug-1) and C-3 (δ_C 74.8). In the HMBC spectrum, correlations were present between the anomeric proton of each sugar and C-4 of the next sugar, establishing the sugar moieties sequence (See Supporting Information). From the above evidences, **1** was identified as 12-benzoylisolineolon-3-*O*-*β*-D-cymaropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D-

oleandropyranosyl-(1→4)-*β*-D-cymaropyranosyl-(1→4)-*β*-D-oleandropyranosyl-(1→4)-*β*-D- oleandropyranosyl-(1→4)-*β*-D-oleandropyranoside and named calotroposide S.

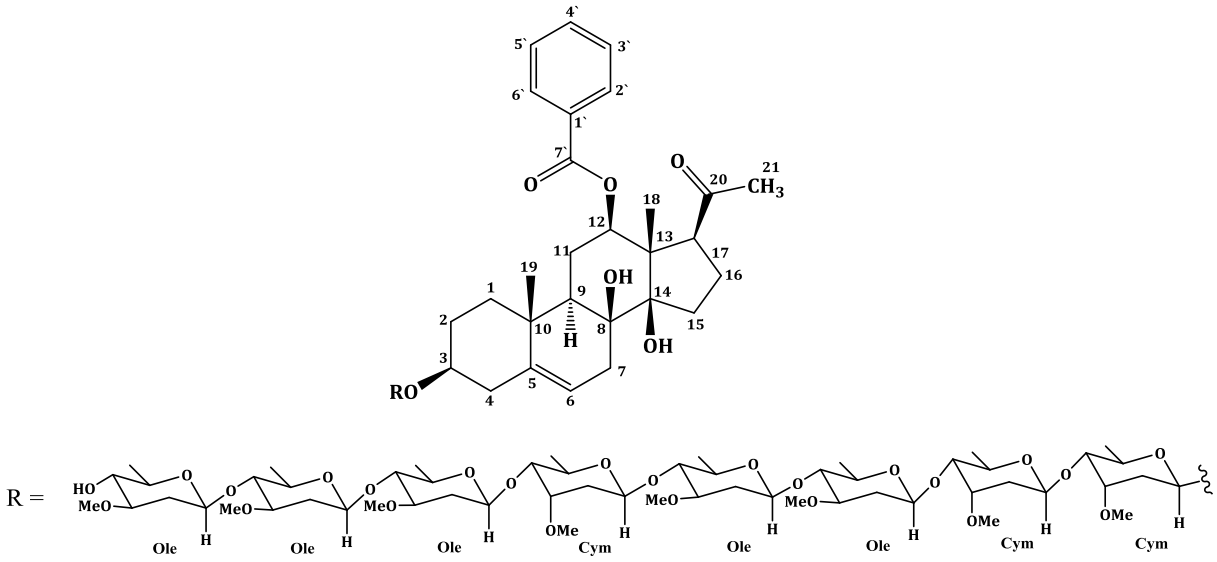

Figure 1. Structure of calotroposide S (**1**).

Compound **1** displayed potent anti-proliferative activity with IC_{50} 0.18, 0.06, and 0.2 μM against PC-3 prostate, U373 (GBM), and A549 (NSCLC) cancer cell lines, respectively compared with cisplatin (IC_{50} 4.0 and 0.4 μM for A549 NSCLC and U373 GBM) and carboplatin (IC_{50} 90.0, 38.0, and >100 μM for the three cancer cell lines, respectively).

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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