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Oligoamide, a new lactam from the leaves of *Angylocalyx oligophyllus*

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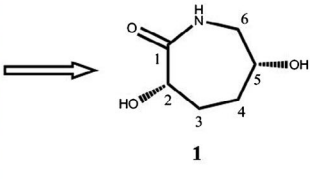
ABSTRACT

A new lactam, oligoamide (**1**), along with three known compounds (**2**–**4**), stigmasterol-3-O-β-D-glucopyranoside (**2**), formononetin (**3**) and (-)-pinitol (**4**) were isolated from the CH_2Cl_2/CH_3OH (1:1) extract of the leaves of *Angylocalyx oligophyllus* by chromatographic separation. Their structures were elucidated on the basis of spectroscopic analysis (UV, IR, MS, 1D, and 2D NMR). Compound **1** was found to have weak antioxidant and urease inhibitory potential.

Angylocalyx oligophyllus



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Angylocalyx oligophyllus; lactam; urease inhibitory activity

1. Introduction

The genus *Angylocalyx* comprises 12 tropical African tree and shrub species, none of which is reported to be poisonous. Many of them have been examined with regard to sugar-mimic alkaloids, polyhydroxylated alkaloids and the previous investigation of *Angylocalyx pynaertii* led to the isolation of 15 polyhydroxylated alkaloids, including the known alkaloids from seeds of this plant, 1,4-dideoxy-1,4-imino-D-arabinitol (DAB), 1-deoxymannojirimycin (DMJ) and 2,5-imino-1,2,5-trideoxy-D-mannitol (6-deoxy-DMDP). Among them, eight sugar-mimic

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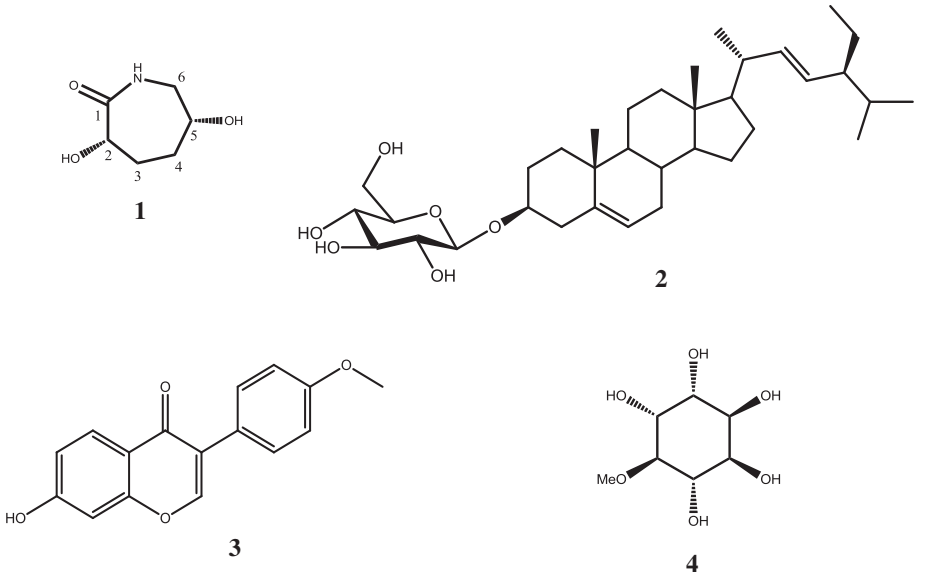

Figure 1. Chemical structures of compounds **1**–**4**.

alkaloids showed the potent inhibitory activity towards bovine epididymis alpha-L-fucosidase (Asano et al. 2001; Nash et al. 1985; Yasuda et al. 2002). Although the alkaloids have been found in both plants and microorganisms, a particularly fruitful source has been the plant family Leguminosae. Many of these alkaloids and structurally related analogues have been synthesized and their glycosidase inhibitory properties evaluated (Pan et al. 1993). Plants of the genus *Angylocalyx* are found in Cameroon at Makenene, Ndikinémeki, Kribi, Mouanko, Song-Mbon and Congo.

As a part of our ongoing research into structurally and biologically interestingsecondary metabolites from Cameroonian plants, a chemical investigation on *Angylocalyx oligophyllus* was undertaken and led to the isolation and characterization of a new lactam, oligoamide (**1**), along with three known compounds identified as, stigmasterol-3-O-β-D-glucopyranoside (**2**) (Manu and Kalia 2013), formononetin (**3**) (Shuhui et al. 2009) and (-)-pinitol (**4**) (Hudlicky et al. 1990). Their structures (Figure 1) were elucidated on the basis of extensive NMR and MS analyses. In addition, the new compound **1** was evaluated for its antioxidant and urease inhibitory activity.

2. Results and discussion

Compound **1** was obtained as a brownish powder. Its molecular formula $C_6H_{11}NO_3$ was determined on the basis of its APCI (+) which showed pseudomolecular ion peak at *m/z* 146.0788 $[M + H]^+$(calcd. 146.0817 for $C_6H_{12}NO_3$), corresponding to two double bond equivalents. The IR spectrum indicated the presence of amide (3287–1604 cm^{-1}) functional group. The ^{1}H-NMR data (Table S1) indicate signals due to two oxymethine protons at δ_H 3.57 and δ_H 3.91 and three methylene groups at δ_H 1.71, 2.25; δ_H 1.55, 2.01; and δ_H 2.79, 3.39. The ^{13}C-NMR spectrum (Figure S2) showed six carbon resonances. Among them, one carbonyl at δ_C 175.0 ppm, two oxygenated methines at δ_C 68.0 (C-5), 62.8 (C-2) and three methylenes

at δ_C 52.0 (C-6), 34.6 (C-4) and 28.5 (C-3). The carbonyl carbon occupying one degree of unsaturation, suggested that compound **1** possessed a monocyclic ring system. In ^{1}H-^{1}H COSY spectrum (Figure S3) cross correlations from H-2/H-3, H-3/H-4, H-4/H-5 and H-5/H-6 allowed the establishment of the long chain C-2/C-3/C-4/C-5/C-6. HMBC correlations observed from H-2 to C-1, C-3 and C-4; H-3 to C-1, C-2 and C4; H-4 to C-2, C-3 and C-5; H-5 to C-4, C-3 and C-6; H-6 to C-4 and C-5 established the seven membered ring of compound **1**. The structure of compound **1** was identified as 3,6-dihydroxyazepan-2-one and has been given the trivial name oligoamide**.** Its relative configuration was determined on the basis of the NOESY correlations (Figure S6) H-2a/H-3e, H-2a/H-6a and H-5e/H-3e.

Compound **1** was subjected to antioxidant and urease inhibitory activity and was found to have moderate activity, 72.9 and 69.1, respectively. The new compound was also evaluated for its glycosidase inhibition effect, but showed no significant activity.

3. Experimental

3.1. General experimental procedures

The melting point was determined on a Barnstead Electro termal apparatus (Digital Melting Point IA-90). Column chromatography was carried out using silica gel (Merck 70–230 and 230–400 mesh). Thin layer chromatography was performed on percolated 0.5 mm thick Merck Si gel 60 F254 aluminium sheets. TLC plates were sprayed with 5% (v/v) aqueous solution of H_2SO_4, ceric sulphate 10% (v/v) and heated at 120 °C on a hot plate to visualize the spots. The mass spectra where recorded on a Compact Bruker MS instrument and nuclear magnetic resonance (NMR) spectra were recorded on a Bruker DPX-400 instrument, ^{1}H and ^{13}C-NMR probes operating at 500 and 125 MHz, respectively with tetramethylsilane as an internal standard.

3.2. Plant material

Leaves of *Angylocalyx Oligophyllus* were collected in April 2014 from Song-Mbon locality, Nyong-et-Kelle Sub-division, Center Region of Cameroon and identified by Victor NANA, a botanist of National Herbarium, Yaoundé, Cameroon; where a voucher specimen is deposited under the voucher number 41503 A. KOUFANI 185.

3.3. Extraction and Isolation

The air-dried and powdered leaves (500 g) of *A. Oligophyllus* was macerated in 7L of dichloromethane/methanol (1/1) for 72 h at room temperature. After filtration and solvent evaporation, a residue of 120 g was obtained. A part of the crude extract (100 g) was subjected to column chromatography (CC) on silica gel employing a step gradient of hexane-ethyl acetate and ethyl acetate-methanol to afford fourteen fractions Fr1-Fr14 based on TLC monitoring. Fraction Fr7 (3.8 g) was purified employing a step gradient of hexane-ethyl acetate and ethyl acetate-methanol to yield stigmasterol-3-*O*-*β*-D-glucopyranoside (**2**) (23.9 mg) formononetin (2.9 mg) (**3**). Fraction Fr10 (9.1 g) eluted with a gradient ethyl acetate-methanol yielded (-)- pinitol (**4**) (57 mg) and oligoamide (**1**) (4.6 mg).

3.4. Antioxidant activity

The free radical scavenging activity was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) using the method described by Gülçin and Aboul-Enein 2007. The solution of DPPH of 0.3 mM was prepared in ethanol. Five microlitres of each sample of different concentration (62.5–500 µg) was mixed with 95 µl of DPPH solution in ethanol. The mixture was dispersed in 96 well plate and incubated at 37° C for 30 min. The absorbance at 515 nm was measured by microtitre plate reader (Spectramax plus 384 Molecular Device, USA) and percent radical scavenging activity was determined in comparison with the methanol treated control. BHA is used as standard. DPPH scavenging effect (%) = $x = \frac{Ac-As}{Ac} \times 100$

Where Ac = Absorbance of Control (DMSO treated), As = Absorbance of Sample

3.5. Urease inhibitory

Reaction mixtures comprising 25 µL of enzyme (Jack bean Urease) solution and 55 µL of buffers containing 100 mM urea were incubated with 5 µL of test compounds (1 mM concentration) at 30 °C for 15 min in 96-well plates. Urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn. Briefly, 45 µL each of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 µL of alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each well. The increasing absorbance at 630 nm was measured after 50 min, using a microplate reader (Molecular Device, USA). All reactions were performed in triplicate in a final volume of 200 µL. The results (change in absorbance per min) were processed by using SoftMax Pro software (Molecular Device, USA). All the assays were performed at pH 8.2 (0.01 M $K_2HPO_4.3H_2O$, 1 mM EDTA.

Disclosure statement

No potential conflict of interest was reported by the authors.

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