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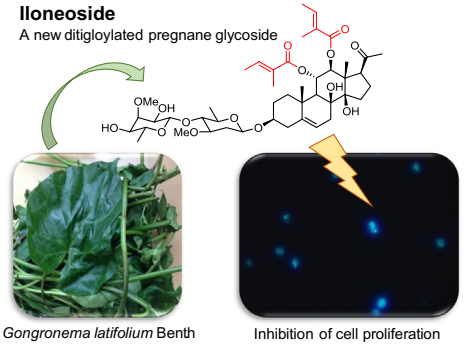
Iloneoside: a cytotoxic ditigloylated pregnane glycoside from the leaves of *Gongronema latifolium* Benth

Gideon Ampoma Gyebi[a], Joseph Oluwatope Adebayo[a], Olufunke Esan Olorundare[b], Antoni Pardede[c,d], Masayuki Ninomiya[c], Afolabi Olanrewaju Saheed[b], Abiola Samuel Babatunde[e] and Mamoru Koketsu[c]

[a]Faculty of Life Science, Department of Biochemistry, University of Ilorin, Ilorin, Nigeria; [b]Faculty of Basic Medical Sciences, Department of Pharmacology and Therapeutics, University of Ilorin, Ilorin, Nigeria; [c]Faculty of Engineering, Department of Chemistry and Biomolecular Science, Gifu University, Gifu, Japan; [d]Department of Chemistry Education, Islamic University of Kalimantan, Banjarmasin, Indonesia; [e]Faculty of Basic Medical Sciences, Department of Haematology, University of Ilorin, Ilorin, Nigeria

ABSTRACT

Gongronema latifolium Benth (*Asclepiadaceae*) is an edible-green-leafy vegetable with known medicinal value. A chemical investigation of the 80% methanolic extract of the leaves led to the isolation of a new pregnane glycoside: iloneoside (3-*O*-[6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→14)-β-D-oleandropyranosyl]-11,12-di-*O*-tigloyl-17β-marsdenin), together with four known constituents. Their chemical structures were determined by spectroscopic analysis. The isolates were tested for their *in vitro* growth inhibitory activity against human leukaemia HL-60 cells. Iloneoside was the most active and gave apoptotic response. Molecular docking analysis demonstrated that iloneoside could be accommodated within hot spots of anti-apoptotic protein Bcl-2. These results suggest *G. latifolium* as a reliable source of potent anticancer compounds.



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CONTACT Mamoru Koketsu koketsu@gifu-u.ac.jp
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1. Introduction

Natural products have over the years been a dependable and inexhaustible source of natural substances for the treatment of various diseases (Newman and Cragg 2012), such as cancer: a second leading cause of death worldwide. Leukaemia is particularly a severe problem in Africa (Fleming 1993). However, for cultural and economic reasons, a number of African people use traditional medicine in conjunction with orthodox medicine. In an African system of medicine, plant-based raw materials are predominantly employed.

Gongronema latifolium Benth (*Asclepiadaceae*) formerly known as *Marsdenia latifolia* is a tropical rain forest herbaceous twiner that produces a yellow flower with a characteristic milky exudates from the stem. *G. latifolium* is traditionally used as spices, vegetables and for various medicinal purposes in the Southeastern and Southwestern parts of Nigeria. Ethnomedical reports have established that various parts of the plant have been used in folklore medicine (Mohammed et al. 2014).

The working hypothesis is bioprospecting of useful molecules for human health, especially anticancer compounds. Herein, we report the isolation, structural elucidation and biological activity of phytochemicals of *G. latifolium* leaves.

2. Results and discussion

An 80% methanolic extract of air-dried *G. latifolium* leaves was successively partitioned with *n*-hexane and EtOAc. The antiproliferative effects of the methanolic extract and the EtOAc fraction on HL-60 cells were investigated using CCK-8 assay (Kakumu et al. 2014). Treatment of the cells with each sample suppressed cell proliferation; in particular, the EtOAc fraction exhibited relatively high inhibition (Figure S1 in Supplementary material). By Hoechst 33,342 staining, the EtOAc fraction caused fragmentation of the nuclei and condensation of the chromatin, both of which are signs of apoptosis, whereas untreated cells had intact nuclei (Figure S2 in Supplementary material).

Compound **1** was an amorphous white powder isolated from the EtOAc fraction and its molecular formula was established as $C_{45}H_{68}O_{15}$ from HRESITOFMS for the peak at *m/z* 847.4477 $[M\text{-}H]^-$ (calcd. for $C_{45}H_{67}O_{15}$ 847.4480). The IR spectrum displayed intense absorption bands of hydroxy (3461 cm^{-1}), carbonyl (1637 cm^{-1}) and olefin (1561 cm^{-1}) functionalities. The ^{13}C NMR and DEPT spectra for the aglycone unit of **1** exhibited a ketone carbonyl group (δ_C 217.2), two ester carbonyl groups (δ_C 168.0 and 166.6), three double bonds (δ_C 139.6, 139.2, 138.3, 128.6, 127.7, 118.2), six methyls, seven methylenes, seven methines and eight quarternary carbons. The ^1H NMR showed three singlet signals (δ_H 2.17, 1.28, 1.24) and an olefinic proton (δ_H 5.41–5.38) that indicated the characteristics of an pregn-5-en-20-one skeleton (Sahu et al. 2002). From the COSY networks observed between δ_H 1.90–1.82 (H-9), 5.91 (H-11), and 4.97 (H-12), it was apparent that C-ring bears two oxygenated functional groups at the C-11 and C-12 positions. These data confirmed the aglycone basic structure to be 3,11,12-tri-*O*-functionalised 17β-marsdenin. The ^1H and ^{13}C NMR suggested the presence of two tigloyl units (Schneider et al. 1993). Their linkages to the 17β-marsdenin skeleton were derived from the HMBC correlations between H-11 and H-12 via an ester bonds to the carbonyl carbons at δ_c 168.0 (C-1′) and 166.6 (C-1′′). The proposed stereochemistry of **1** (Figure S3 in Supplementary material) was based on dipolar interactions observed in the

ROESY spectrum (Efdi et al. 2012). Therefore, the aglycone was established as 3-*O*-functionalyzed 11,12-di-*O*-tigloyl-17β-marsdenin (Deng et al. 2005).

After subtracting the elemental composition of the aglycone from the molecular formula of compound **1**, $C_{14}H_{23}O_5$ was left. The ^{1}H NMR spectrum of compound **1** displayed two anomeric proton signals at δ_H 4.79 and 4.56 with corresponding carbons at δ_C 99.2 and 97.3, suggesting the sugar moiety consist of two units. By comparing our spectra data with those reported from the same subfamily (Schneider et al. 1993; Sahu et al. 2002; Deng et al. 2005), we assigned the inner sugar to be oleandropyranose (Ole) and the outer sugar to be 6-deoxy-3-methylallose (Meall). From the HMBC spectrum, the correlation of anomeric proton of Meall at δ_H 4.79 to δ_C 79.2 (Ole^{C-4}) suggested a (1→4) glycosidic linkage, while that of Ole at δ_H 4.56 correlated with δ_c 77.9 (C-3) of the aglycone. Thus, compound **1** was identified as 3-*O*-[6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→14)-β-D-oleandropyranosyl]-11,12-di-*O*-tigloyl-17β-marsdenin. To the best of our knowledge, compound **1** represents a previously unreported pregnane glycoside that we named iloneoside, because the iloneoside was isolated from the plant obtained from Ilorin, Nigeria (Figure 1). By comparing our data with those reported previously (Yoshimura et al. 1985; Schneider et al. 1993; Morvai et al. 2000), we determined compound **2** as marsectohexol, **3** as 3-*O*-[6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-oleandropyranosyl]-17β-marsdenin, **4** as 3-*O*-[6-deoxy-3-*O*-methyl-β-D-allopyranosyl-(1→4)-β-D-canaropyranosyl]-17β-marsdenin and **5** as ajugoside, respectively. Their physical data are given in Supplementary material.

The antiproliferative effects of compounds **1**–**5** on HL-60 cells were investigated. All isolated compounds inhibited cell proliferation at a concentration of 50 μM (**1**: 4.7 ± 0.3%; **2**: 22.3 ± 1.2%; **3**: 23.6 ± 3.7%; **4**: 27.6 ± 1.0%; **5**: 18.4 ± 0.4%, % of control), and the IC_{50} value of **1** was 25.8 μM. Moreover, we studied the morphological alteration in the cultured cells treated with the isolates **1** and **3** at a concentration of 50 μM by staining analysis. The Hoechst 33342-stained microscopic images are shown in Figure S4 in Supplementary material. Iloneoside (**1**)-treated cells showed nuclei fragmentation, chromatin condensation and membrane blebbing, which are morphological features of apoptosis.

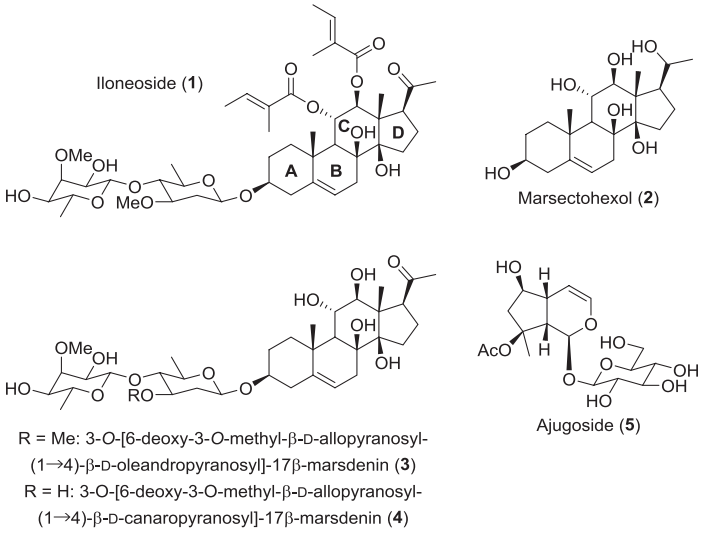

Figure 1. Chemical structures of isolated compounds.

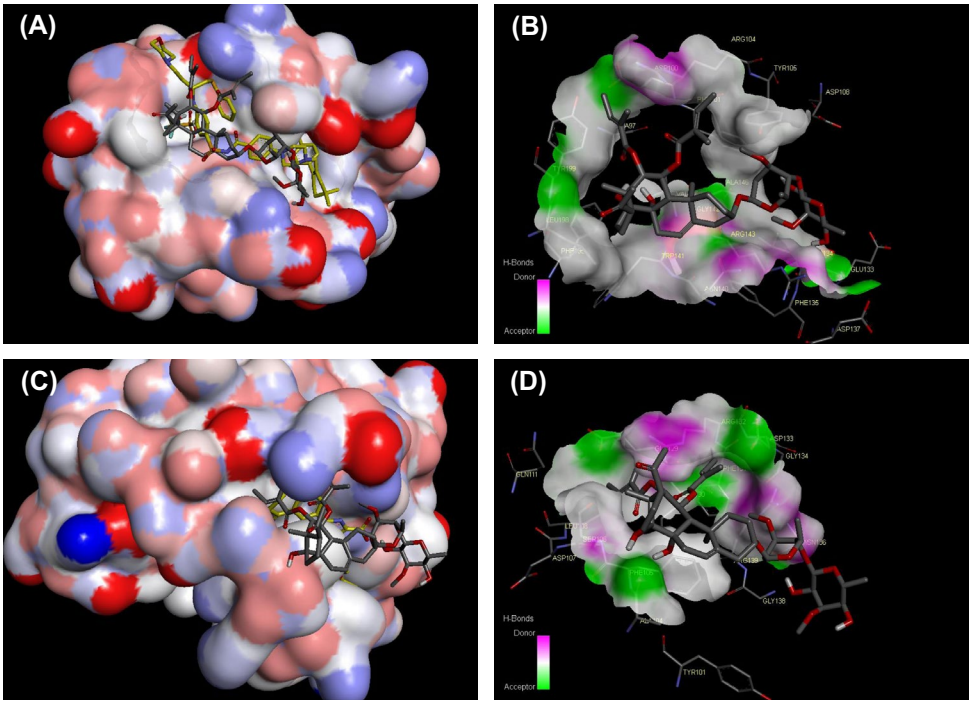

Figure 2. Molecular docking analysis of iloneoside (**1**) to Bcl-2 and Bcl-xL: (A) Surface view of Bcl-2 binding **1** and navitoclax (carbon atoms coloured yellow); (B) 3D-Orientation of **1** in navitoclax binding site of Bcl-2; (C) Surface view of Bcl-xL binding **1** and WEHI-539 (carbon atoms coloured yellow); and (D) 3D-Orientation of **1** in WEHI-539 binding site of Bcl-xL.

The Bcl-2 family of proteins includes both anti- and pro-apoptotic proteins, and maintains the delicate balance between cell death and cell survival (Cory and Adams 2002; Ola et al. 2011). Hence, we explored the detailed intermolecular interactions and probable binding mode of iloneoside (**1**) with anti-apoptotic proteins Bcl-2 (PDB ID: 4LVT) and Bcl-xL (PDB ID: 3ZLR) by computational docking simulations. Free binding energies of the best pose of **1** in Bcl-2 and Bcl-xL were ΔG −10.41 and −7.75 kcal/mol, respectively. Iloneoside (**1**) favourably docked to the navitoclax (an inhibitor) binding hot spots of Bcl-2 (Figure 2(A)). Two tigloyl groups were close to the residues Ala97, Asp100, Phe101, Arg104, Tyr105, Asp108 and Tyr199 (Figure 2(B)), and the 12-*O*-tigloyl moiety displayed the hydrophobic contact with Tyr199. In addition, **1** inserted two tigloyl units into the cleft formed by Ser106, Leu108, Glu129, Arg132 and Asp133 (WEHI-539 binding residues) of Bcl-xL and the steride basic skeleton was arranged in the outside of binding regions (Figure 2(C) and 2(D)). These data implied that two tigloyl units were responsible for binding affinity and specificity to Bcl-2 and Bcl-xL.

3. Conclusion

Our chemical investigation of *G. latifolium* leaves resulted in the discovery of a new cytotoxic ditigloylated pregnane glycoside (iloneoside). These results provide the scientific information and enhance the ethnopharmacological value of *G. latifolium* leaves.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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ORCID

Mamoru Koketsu http://orcid.org/0000-0002-1496-2177

References

Cory S, Adams JM. 2002. The bcl2 family: regulators of the cellular life-or-death switch. Nat Rev Cancer. 2:647–656.

Deng J, Liao Z, Chen D. 2005. Marsdenosides A-H, polyoxypregnane glycosides from *Marsdenia tenacissima*. Phytochemistry. 66:1040–1051.

Efdi M, Ninomiya M, Suryani E, Tanaka K, Ibrahim S, Watanabe K, Koketsu M. 2012. Sentulic acid: a cytotoxic ring A-*seco* triterpenoid from *Sandoricum koetjape* Merr. Bioorg Med Chem Lett. 22:4242–4245.

Fleming AF. 1993. Leukaemias in Africa. Leukemia. 7:138–141.

Kakumu A, Ninomiya M, Efdi M, Adfa M, Hayashi M, Tanaka K, Koketsu M. 2014. Phytochemical analysis and antileukemic activity of polyphenolic constituents of *Toona sinensis*. Bioorg Med Chem Lett. 24:4286–4290.

Mohammed A, Ibrahim MA, Islam MS. 2014. African medicinal plants with antidiabetic potentials: a review. Planta Med. 80:354–377.

Morvai M, Nagy T, Kocsis A, Szabo Laszlo F, Podanyi B. 2000. Effect of oxygen substituents on two- and three-bond carbon-proton spin-spin coupling constants. Magn Reson Chem. 38:343–359.

Newman DJ, Cragg GM. 2012. Natural Products As Sources of New Drugs over the 30 Years from 1981 to 2010. J Nat Prod. 75:311–335.

Ola MS, Nawaz M, Ahsan H. 2011. Role of Bcl-2 family proteins and caspases in the regulation of apoptosis. Mol Cell Biochem. 351:41–58.

Sahu NP, Panda N, Mandal N, Banerjee S, Koike K, Nikaido T. 2002. Polyoxypregnane glycosides from the flowers of *Dregea volubilis*. Phytochemistry. 61:383–388.

Schneider C, Rotscheidt K, Breitmaier E. 1993. Four new pregnane glycosides from *Gongronema latifolium* (Asclepiadaceae). Liebigs Ann Chem. 1057–1062.

Yoshimura S, Narita H, Hayashi K, Mitsuhashi H. 1985. Studies on the constituents of Asclepiadaceae plants. LIX. The structures of five new glycosides from *Dregea volubilis* (L.) Benth. Chem Pharm Bull. 33:2287–2293.