

A New Cytotoxic Ceramide from *Dalbergia boehmii* Taub. Stem Bark

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ABSTRACT

Background: *Dalbergia boehmii* (Leguminosae), is a medicinal plant used in the Northern part of Cameroon, against digestive tract diseases and fever. **Objectives:** This work which is part of the search for bioactive molecules through natural products, aimed to isolate, characterize and evaluate the cytotoxicity of compounds from *D. boehmii*. **Materials and Methods:** Chemical investigations were carrying out on stem barks of *Dalbergia boehmii* using chromatographic techniques. Structures elucidation of isolated compounds were done using 1D and 2D NMR as well as EI-MS and literature data. Some isolated compounds were evaluated *in vitro* against MCF-7 cell lines for their cytotoxicity activity, using the MTT colorimetric method. **Results:** The chemical investigation led to the isolation of a new ceramide derivative named Dalbergiamide (1), a triterpene identified as olean-12-ene-3,11-dione (2) isolated here for the first time from *Dalbergia* genus, along with two known compounds, glucoside 3-O-β-D-sitosterol (3) and glucoside 3-O-β-D-stigmasterol (4). Compounds 1 and 2 were found to be cytotoxic at 50 μM with IC₅₀ value of 17.13 μM and 35.52 ± 2.95 μM respectively. **Conclusion:** These promising results suggested more in-depth studies should be carried out on this plant in order to assess its potential in the fight against cancer.

Keywords: *Dalbergia boehmii*, Natural products, Cytotoxicity, MCF-7.

INTRODUCTION

Dalbergia species belonging to Leguminosae family, is very appreciated in wood crafts because of the variety of pigmented colors of their wood, are also valued for their use in traditional medicine.^[1] *Dalbergia* species are also known to possess several pharmacological activities including antiplasmodial, anti-inflammatory, analgesic, antimicrobial, antioxidant, antiulcerogenic, antipyretic, anti-seizure, cytotoxic and cancer chemopreventive activities.^[2-4] Phytochemical studies showed that plants from *Dalbergia* genus contain various classes of compounds as phenolic compounds, stibenes, terpenoids, glycosides, miscellaneous, ...etc.^[5,6]

As part of our continued screening program of Cameroon medicinal plant constituents especially *Dalbergia boehmii*, we carried out investigation on the stem barks. *Dalbergia boehmii* which is known as *Ngalayhi* in *Fulfulde* in the Northern Cameroon, is a small to medium-sized deciduous tree or a shrub, used traditionally to treat digestive tract diseases and fever.^[6,7] Previous chemical investigation carried on leaves and hearthwood of *D. boehmii* led to the isolation of thirteen phenolic compounds, mostly isoflavonoids and stilbenes.^[6] Further to this, the phytochemical study of the stem barks gave four compounds including a new ceramide named dalbergiamide, a triterpene: olean-12-ene-3,11-dione and two phytosterols:

glucoside 3-O-β-D-sitosterol and glucoside 3-O-β-D-stigmasterol. Antiproliferative activity against the human breast cancer cell lines (MCF-7) of some isolated compounds has been evaluated.

Experimental Section

General experimental procedures

¹H NMR and ¹³C NMR spectra were recorded on a Bruker AM 400 (600) and AMX 500 NMR (Avance) spectrometer respectively at 500 MHz and 125 MHz using pyridine-*d*₅ and CDCl₃ as solvents. JEOL JMS-600H spectrometer was used for EI-MS spectra recording; TMS was used as internal standard and chemical shifts are in ppm. Silica gel was used for column chromatography (CC) monitored by TLC on alumina precoated silica gel sheets (60F₂₅₄). Spots were visualized using UV lamp (254 and 386 nm) followed by spraying ceric sulphate solution and heating at 105°C.

Plant material

Identified by Dr Tsabang Nole in Touboro in the North region of Cameroon, stem barks of *Dalbergia boehmii* were collected in September 2012, and

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authenticated at the Cameroon National Herbarium where a Voucher specimen (66887/HNC) has been deposited.

Extraction and purification

The pulverized barks (3 kg) was macerated in 9 L of a mixture of $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (1:1) at room temperature, to give after filtration and solvent evaporation under reduced pressure 159.7 g of a brown crude extract. Silica gel column chromatography (CC) of 150.0 g of this crude extract using a gradient of Hexane-EtOAc (1 \rightarrow 0 to 0 \rightarrow 1) then EtOAc-MeOH (1 \rightarrow 0 to 0 \rightarrow 1) was performed. 210 fractions were collected and pooled according to their TLC profiles leading nine fractions (A-I). The purification of A (6.0 g) on silica gel CC furnished 15.0 mg of compound 2 using a mixture of Hexane-EtOAc (9:1) as eluent. Fraction I (8.0 g) was also subjected to CC of silica gel eluted with EtOAc (100%) yielding 20.0 mg of a mixture of compound 3 and 4 while fraction B furnished compound 1 (2.0 mg) when eluted with Hexane- EtOAc (8:2).

Dalbergiamide (1): White amorphous powder; m.p. 129-131°C; EI-MS: m/z 667.2 calculated for $\text{C}_{41}\text{H}_{81}\text{O}_5\text{N}$. $^1\text{H-NMR}$ (500 MHz, Pyr- d_5): δ_{H} 0.87 (6H, t, 6.9, H-21, H-20'), 1.20-1.34 (52H, m, H-7-20; H-4'-13' and H-18'-19'), 1.70 (2H, m, H-6), 1.92 (1H, m, H-5b), 1.98 (2H, m, H-14'), 2.03 (1H, m, H-3'b), 2.16 (2H, m, H-17'), 2.22 (1H, m, H-3'a), 2.26 (1H, m, H-5b), 4.29 (1H, m, H-4), 4.36 (1H, m, H-3), 4.42 (1H, m, H-1a), 4.51 (1H, m, H-1b), 4.61 (1H, dd; 10.4; 6.5, H-2'), 5.12 (1H, m, H-2), 5.49 (1H, m, H-16'), 5.54 (1H, m, H-15'), 6.21 (brs, OH-4), 6.68 (2H, brs, OH-1 and OH-3), 7.60 (brs, OH-2'), 8.57 (1H, d, 9.0, NH). $^{13}\text{C-NMR}$ (75 MHz, Pyr- d_5): δ_{C} 14.3 (C-21, C-20'), 22.9 (C-20, C-19'), 25.8 (C-6), 26.7 (C-4'), 29.5-30.2 (C-7-18, C-5'-13'), 32.1 (C-19, C-18'), 32.9 (C-14'), 33.3 (C-17'), 34.2 (C-5), 35.7 (C-3'), 53.0 (C-2), 62.1 (C-1), 72.5 (C-2'), 73.1 (C-4), 76.8 (C-3), 130.7 (C-15'), 130.8 (C-16'), 175.2 (C-1').

Cytotoxicity assay

Cytotoxicity activity of isolated compounds 1 and 2 was evaluated *in vitro* against MCF-7 cell lines using the MTT colorimetric standard method according to Scudiere *et al.*^[8]

RESULTS

Chromatographic separations of the crude extract of the stem barks of *D. boehmii* obtained by extraction with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (1:1), led to a new ceramide: dalbergiamide (1) along with one known triterpene and a mixture of phytosterol glucosides. The known triterpene was identified as olean-12-ene-3,11-dione (2)^[9] and the mixture of steroid glucosides as glucoside 3-*O*- β -D-sitosterol (3) and glucoside 3-*O*- β -D-stigmasterol (4)^[10] (Figure 1) comparing their spectroscopic data with those found in literature.

In vitro cytotoxicity activity of dalbergiamide (1) and olean-12-ene-3,11-dione (2) was evaluated against MCF-7 cell lines using MTT colorimetric method, and were found to be active with IC_{50} value 17.13 μM and 35.52 \pm 2.95 μM respectively when tested at the concentration of 50 μM .

DISCUSSION

Compound 1 was isolated for the first time from natural sources, and its structure has been elucidated as follows: obtained as a white solid (m.p. 129-131°C), its molecular formula $\text{C}_{41}\text{H}_{81}\text{O}_5\text{N}$ was determined from rigorous analysis of its NMR spectra (1D & 2D) and its EI-MS spectrum which exhibited molecular ion peak $[\text{M}]^+$ at m/z 667.2 and characteristic fragments ions at m/z 647.2 $[\text{M}-\text{H}_2\text{O}-\text{H}_2]^+$, 619.4 $[\text{M}-\text{H}_2\text{O}-\text{H}_2-\text{CO}]^+$. The 1D NMR spectra of this compound, shows characteristic signals, of a ceramide backbone, with generally an amide function and two long aliphatic chains, more or less unsaturated.^[11-15] In the ^1H NMR spectrum of compound 1, a signal of a proton exchangeable with D_2O is observed at δ_{H} 8.57 (d, $J = 9.0$ Hz) advising the presence of the group -NH-CO-

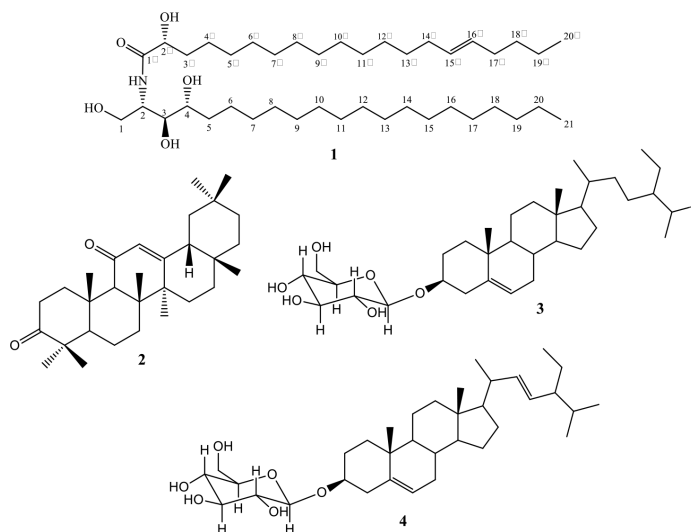


Figure 1: Structures of isolated compounds (1-4).

Moreover, $^{13}\text{C-NMR}$ spectrum showed at δ_{C} 53.0 a signal of C-N and a C=O signal at δ_{C} 175.2 confirming the presence of a secondary amide function in this compound.^[15,16] ^1H NMR spectrum also exhibited resonances for terminal methyls of two aliphatic chains at δ_{H} 0.87 (6H, t, $J = 6.9$ Hz), an azomethine proton at δ_{H} 5.12 (1H, m), signals at δ_{H} 4.51 (m) and 4.42 (m) for an hydroxymethylene group, three carbinylic protons at δ_{H} 4.29 (H-4), 4.36 (m, H-3) and 4.61 (m, H-2'), as well as signals of four -OH groups at δ_{H} 6.21 (1H, brs), 6.68 (2H, brs) and 7.68 (brs). Two alkyl long chains were also observed as a broad signal at δ_{H} 1.20-1.34. Further extensive analysis of the ^1H NMR spectrum, reveals signals of two olefinic protons at δ_{H} 5.54 (m) and 5.49 (m), suggesting the presence of a disubstituted double bond. As expected, instead the characteristic signals of secondary amide group, the ^{13}C NMR spectrum of compound 1 exhibited two carbon signals at δ_{C} 130.8 and 130.7 corresponding to a double bond, with a *trans* configuration, that was determined by the chemical shifts of its allylic carbon atoms appearing at δ_{C} 32.9 and 33.3.^[17,18] This double bond was located in the fatty acid moiety at C-15' by EI-MS analysis. Signals of an oxymethylene at δ_{C} 62.1 and three oxymethines at δ_{C} 76.8, 73.1 and 72.5 were also visible. Additionally, several signals of carbon atoms in the range of δ_{C} 34.2-22.9 related to - CH_2 - groups and a signal at δ_{C} 14.3 corresponding to two terminals - CH_3 were also deduced from ^{13}C NMR spectrum, confirming that compound 1 belongs to the class of ceramide.

In the ^1H - ^1H COSY spectrum several correlations are observed. It is the case of the correlation of the two - CH_2 - protons at δ_{H} 4.42 and 4.51 with the methine proton at δ_{H} 5.12, which also showed correlation with the methine at δ_{H} 4.36. This last methine proton at δ_{H} 4.36 also correlated with the methine proton at δ_{H} 4.29, suggesting three hydroxyl groups respectively on C-1, C-3 and C-4. This spectrum also exhibited correlations between the two olefinic protons at δ_{H} 5.54 and 5.49, as well as, respective correlations between these protons and their adjacent methylenes at δ_{H} 1.98 and 2.16. The correlation of H-2' at δ_{H} 4.61 and the methylene protons H-3' at δ_{H} 2.03 and 2.22 was also visible. Interpretation of the HMBC spectrum revealed correlations of the proton of amide group at δ_{H} 8.57 and -C=O group at δ_{C} 175.2 and the -CH-NH at δ_{C} 53.0; correlations of H-2 (δ_{H} 5.12) and C-1 (δ_{C} 62.1) and C-3 (δ_{C} 76.8); correlations of H-3 (δ_{H} 4.36) and C-1 (δ_{C} 62.1), C-2 (δ_{C} 53.0), and C-4 (δ_{C} 73.1), confirming positions of -OH groups on C-1, C-3 and C-4 (Figure 2).^[15] Correlations between H-2' (δ_{H} 4.61) -C=O (δ_{C} 175.2) and

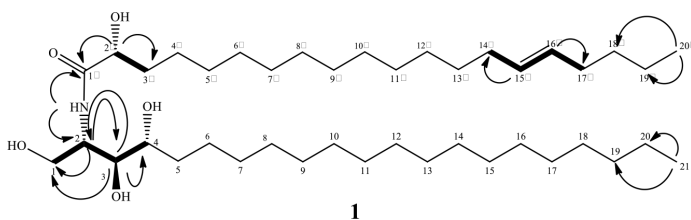


Figure 2: ^1H - ^1H COSY (—) and key HMBC correlations ($\text{H} \rightarrow \text{C}$) of dalbergiamide (1).

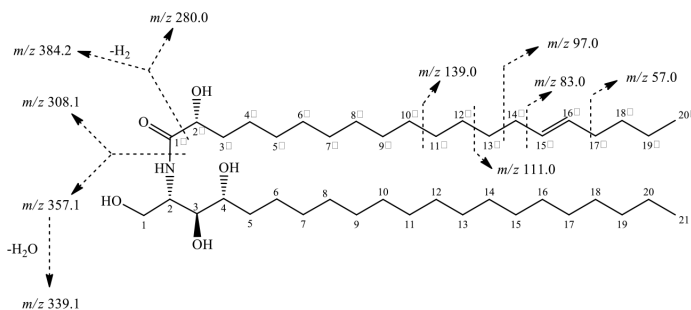


Figure 3: EI-MS fragmentation pattern of dalbergiamide (1).

C-3' (δ_{C} 35.7) were also visible confirming the α -hydroxy fatty acid side chain.

The length of the long chain base and the fatty acid was determined by EI-MS, which showed significant fragment ion peaks at m/z 384, 357 and 339 that were congruent to the long chain base moiety (Figure 3). The length of the fatty acid chain was deduced from the fragment at m/z 280 resulting from the cleavage in α of the carbonyl group of the amide function. This fragment, combined with the ones observed at m/z 57 and 83, resulting from the α -cleavages of the double bond, confirm the emplacement of this olefine bond on the fatty acid chain. All these assignments were congruent with the proposed structure.

Finally, considering the steric hindrance and biogenesis of sphingolipids, absolute stereochemistry of the phytosphingosine moiety can be generally determine using chemical shift of the carbon C-2 to C-4.^[17] Thus, according to the ^{13}C NMR data of compound **1**, the relative stereochemistries of C-2 (δ_{C} 53.0), C-3 (δ_{C} 76.8), and C-4 (δ_{C} 73.1), were found to be 2*S*, 3*S*, and 4*R*.^[17,18] From the above analysis the structure of compound **1** was set as (2'*R*, 15'*E*)-2'-hydroxy-N-[(2*S*,3*S*,4*R*)-1,3,4-trihydroxyhenicosan-2-yl]eicos-15'-enamide named dalbergiamide.

The new compound dalbergiamide (**1**) and the triterpene olean-12-ene-3,11-dione (**2**) were tested for their cytotoxic activities against MCF-7 cells. These two compounds were slightly active at 50 μM , the most remarkable cytotoxic activity against breast cancer cells MCF-7 being recorded for compound **1** with IC_{50} value of $17.13 \pm 0.21 \mu\text{M}$ while compound **2** was less active with IC_{50} value $35.52 \pm 2.95 \mu\text{M}$. Previous studies have proved the activity of ceramides against cancer cell lines^[19] and established their mechanism of action. Ceramides have been shown to act by blocking the cell cycle, following the up-regulation of p27^{kip1} thus causing apoptosis.^[20] This could explain why the new ceramide, dalbergiamide (**1**) showed promising cytotoxic activity against MCF-7 cancer cell lines. The results also corroborate with those of some authors who showed that one of the most important biological effects of natural terpenoids is the ability to prevent and to treat several cancers including breast cancer.^[21-25] Others studies have shown that triterpenes with an oleanane type skeleton displayed cytotoxic activity against cancer cell lines.^[16]

CONCLUSION

The crude $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (1:1) extract of *D. boehmii* stem barks afforded a new ceramide named dalbergiamide (**1**); a triterpene: olean-12-ene-3,11-dione (**2**) and a mixture of two sterols: glucoside 3-O- β -D-sitosterol (**3**) and glucoside 3-O- β -D-stigmasterol (**4**). The new ceramide dalbergiamide (**1**) and the triterpene olean-12-ene-3,11-dione (**2**) showed cytotoxic activity against MCF-7 cancer cell lines, the most remarkable activity being recorded for compound **1**. This result, associated with previous, suggests that a particular intention should be placed on this plant in order to evaluate its action in the treatment of cancers.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

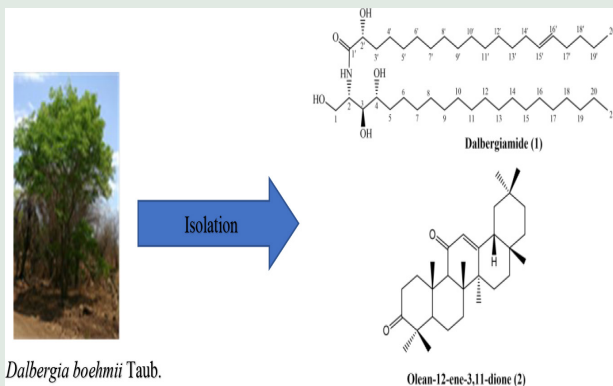
NMR: Nuclear Magnetic Resonance; **HMBC:** Heteronuclear Multiple Bonds Correlation; **COSY:** CORrelated Spectroscopy; **HSQC:** Heteronuclear Single Quantum Correlation; **EI-MS:** Electronic Impact Mass Spectrometry; **CH_2Cl_2 :** Methylene Chloride; **MeOH:** Methanol; **TLC:** Thin Layer Chromatography; **EtOAc:** Ethyl acetate; **brs:** broad singlet; **m:** multiplet; **dd:** doublet doublet; **DMSO:** Dimethyl sulfoxide; **MTT:** 3-[4,5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide, **OD:** Optical Density.

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GRAPHICAL ABSTRACT



SUMMARY

- Chemical constituents of *Dalbergia boehmii* Taub. were investigated
- *In vitro* cytotoxicity activity of some isolated compounds was investigated against MCF-7 cell lines.
- Compounds 1 and 2 were found to be cytotoxic at 50 μM with IC_{50} value 17.13 μM and $35.52 \pm 2.95 \mu\text{M}$ respectively
- *Dalbergia boehmii* Taub. is a potential source of cytotoxic agents.

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