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Phytochemical study of the stem bark of *Tetrorchidium didymostemon* (Euphorbiaceae)

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Abstract
This work deals with the phytochemical study of the stem bark of *T. didymostemon*, a plant used in the Gabonese traditional medicine in the treatment of various ailments. A chromatographic separation of the dichloromethane-methanol (1:1) extract of this plant part led to the isolation of four compounds, that were identified respectively to 1-hentriacontanol 1, stigmasta-5,24,28-trien-3-ol 2, stigmasta-5,24,28-trien-3-O-β-D-glucopyranoside 3 and tithoniamide B 4. Their structures were elucidated on the basis of common spectroscopic analysis techniques and by comparison of their spectral and physical data with those from the literature. From a chemotaxonomic point of view, these compounds are described from *T. didymostemon* for the first time.

Keywords: Phytochemistry; *T. didymostemon*; Chromatographic separation; Secondary metabolites; characterization

1. Introduction
Tetrorchidium didymostemon (Baill.) Pax & K. Hoffm.(*Euphorbiaceae*) is an evergreen shrub or a tree that grows in many parts of the tropical Africa, from Guinea Bissau to Central African Republic, including Cameroon, Equatorial Guinea, Gabon, Congo, DR Congo, Uganda, Tanzania and Angola. The plant can reach up to 25 m tall, medium in size, with hanging branches. The bole can be 60 cm or more in diameter [1, 2].
T. didymostemon is widely used in the African folk medicine in the treatment of filariasis, abscesses, leprous sores, glandular swellings, malaria, oedema, purgative enema, rheumatic painful limbs, painful kidney, toothache, and also as snakebites antidote, diuretic, emetic, febrifuge, parasiticide and purgative [3-5].
In a previous work [6], *T. didymostemon* extracts exhibited good antimicrobial activities and high biomolecules content, including alkaloids, flavonoids, tannins, triterpenes, polyphenols and sterols [6].
In the present paper, we report the isolation and structural determination of four compounds from the stem bark of *T. didymostemon*, through the chromatographic fractionation of the dichloromethane-methanol extract of this plant part, and the analysis of the spectroscopic data of the biomolecules.

2. Materials and Method
2.1. Plant material: Stem bark (2.5 kg) of *T. didymostemon* was harvested near Franceville (Gabon), and a specimen was kept in our university (N° Td 066/UM).

2.2. Extraction and Isolation
The plant material was dried for three weeks at room temperature and finely powdered. Powder obtained was extracted with a dichloromethane-methanol (1:1) mixture for three days and the extract was freeze-dried to yield 150 g of stem bark extract.
The extract (12 g) was subjected to repeated flash column chromatography over silica gel (70-230 mesh) columns, and eluted successively with n-hexane and n-hexane-ethylacetate mixtures of increasing polarities. The fractions were checked by TLC and those of similar contents were combined and concentrated. This yielded: 1-hentriacontanol 1 (51 mg) [n-hexane/ethylacetate (97/03)], stigmasta-5,24,28-trien-3-ol 2 (114.1 mg) [n-hexane/ethylacetate (90/10)], stigmasta-5,24,28-trien-3-O-β-D-glucopyranoside 3 (24.49 mg) [n-hexane/ethylacetate (70:30)] and tithoniamide B 4 (19,91 mg) [n-hexane/ethylacetate (65:35)] (Fig. 1).

2.3. Physical and Spectral Data of the Compounds (1, 2, 3 and 4)

2.3.1. Compound 1: white powder; m.p. 84-86 °C; molecular formula: $C_{31}H_{62}O$. M (EI): m/z = 452 $[M]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ_H (ppm) 3.62 (2H, t, *J* = 6.8 Hz); 1.54 (2H, m); 1.26 (58H, bs); 0.86 (3H, d, *J* = 6.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm) 63.12; 32.83; 31.93; 29.66; 29.51; 29.44; 25.75; 22.69; 14.10.

2.3.2. Compound 2: white crystals; m.p. 152-154° C; molecular formula: $C_{29}H_{46}O$. M (EI): m/z = 410 $[M]^+$. ^1H NMR (400 MHz, $CDCl_3$) δ_H (ppm) 5.32 (1H, bs), 5.22 (1H, m), 5.16 (1H, m), 4.68 (2H, bs), 3.50 (1H, m), 1.63 (3H, s), 1.01 (3H, d, *J* = 5.2 Hz), 0.99 (3H, s), 0.81 (3H, t, *J* = 6 Hz), 0.67 (3H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm) 148.62, 140.76, 137.19, 130.04, 121.69, 109.51, 71.80, 56.85, 55.89, 51.99, 50.16, 42.31, 40.17, 39.68, 37.26, 36.52, 31.90, 31.67, 28.69, 25.71, 24.32, 21.07, 20.79, 20.21, 19.39, 12.31, 12.05.

2.3.3. Compound 3: beige powder; molecular formula: $C_{35}H_{56}0_6$. M (EI): m/z = 572 $[M]^+$. ^1H NMR (400 MHz, Pyr-D5) δ_H (ppm) 5.34 (1H, m), 5.30 (2H, t, *J* = 6.4 Hz), 5.05 (2H, d, *J* = 7.6 Hz), 4.85 (2H, d, *J* = 5.6 Hz), 4.57 (1H, m), 3.95 (2H, bs), 1.71 (3H, s), 1.04 (3H, d, *J* = 6.4 Hz), 0.91 (3H, s), 0.87 (3H, t, *J* = 7.2 Hz), 0.65 (3H, s). ^{13}C NMR (125 MHz, Pyr-D_5) δ_C (ppm) 148.64, 140.95, 137.61, 130.34, 121.91, 110.18, 102.60, 78.51, 78.12, 75.38, 71.75, 62.88, 56.91, 56.03, 52.30, 50.37, 42.39, 40.48, 39.84, 37.50, 36.96, 32.07, 29.07, 26.07, 24.51, 21.28, 20.32, 12.40.

2.3.4. Compound 4: white powder; m.p. 134-136 °C; molecular formula: $C_{42}H_{83}NO_5$. M (FAB): m/z = 680.4 $[M-H]^+$. UV-vis: λ_{max} = 229, 263 nm. IR (KBr): υ = 3693, 3414, 2921, 2851, 1627 cm^{-1}. ^1H NMR (500 MHz, Pyr-D_5) δ_H 8.57 (1H, d, *J* = 8.8 Hz), 5.55 (1H, m), 5.49 (1H, m), 5.12 (1H, bs), 4.62 (1H, bs), 4.50 (1H, bs), 4.43 (1H, bs), 4.35 (1H, bs), 4.29 (1H, bs), 0.85 (6H, t, *J* = 6.8 Hz). ^{13}C NMR (125 MHz, Pyr-D_5) δ (ppm) 175.25, 130.82, 130.70, 76.86, 73.03, 62.04, 52.99, 35.72, 34.16, 33.84, 33.30, 32.98, 32.12, 30.34, 30.18, 30.00, 29.92, 29.87, 29.82, 29.62, 29.60, 29.51, 26.72, 26.66, 25.82, 22.93, 14.27.

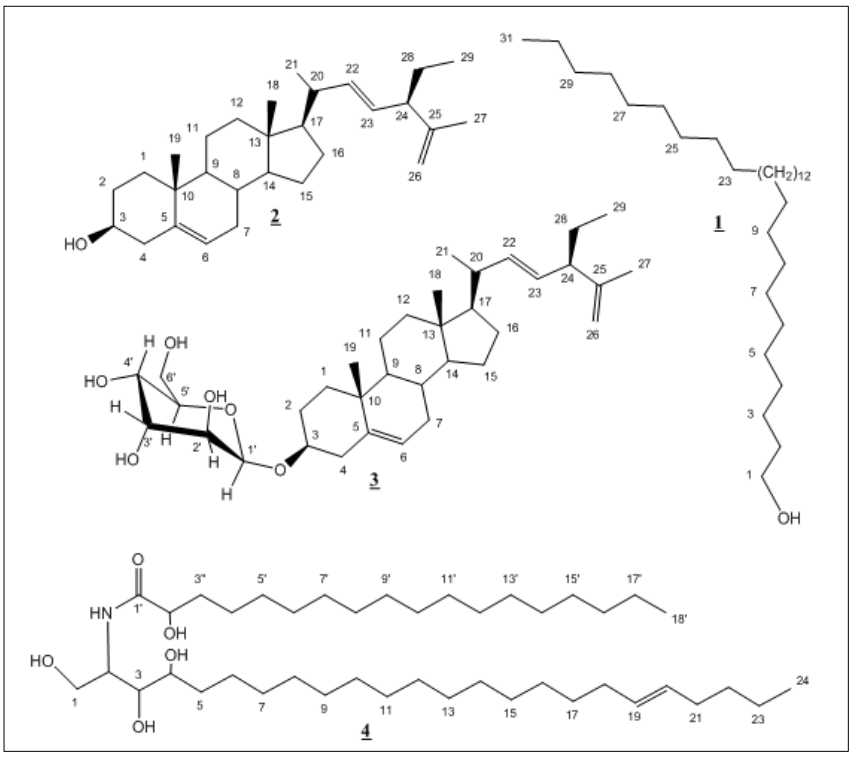

Fig 1: Structure of compounds 1 - 4 isolated from *T. didymostemon*

3. Results and Discussion

3.1 Identification of isolated compounds

Compound 1 was isolated as a white powder soluble in chloroform. Its mass spectrum showed a molecular ion at m/z 452, corresponding to the formula $C_{34}H_{62}O$ with 0 degree of unsaturation.

Its ^1H NMR spectrum showed a peak at δ_H 3.62, integrating for two protons, assignable to an oxymethylene group. This spectrum also showed a set of peaks between δ_H 1.15 and 1.45 which are those of a long chain of alkylpolymethylenes, and a terminal methyl at δ_H 0.86; thus suggesting the lipid nature of 1. The completely decoupled ^{13}C NMR spectrum of 1, as well as that of DEPT 135 revealed the presence of 1 methyl group and 30 methylenes groups. The ^{13}C NMR spectrum also showed a peak at δ_C 63.12 that could be assigned to a methylene linked to a hydroxyl group.

A comparison between the spectroscopic data of 1 with those found in the literature [7, 8] allows us to assign to compound 1 the structure 1 which is that of 1-Hentriacontanol [7, 8] (Fig. 1).

Compound 2 was obtained as white crystals, with a melting point between 152-154 °C. It was soluble in chloroform and responded positively to the Liebermann-Burchard test, showing a violet colour of sterols. Its ^1H NMR spectrum showed a set of 5 intense peaks between δ_H 0.60 and 1.70 assignable to five methyl groups. This suggested the tetracyclic nature of 2. This spectrum also showed five remarkable peaks at δ_H 3.50, 4.68, 5.16, 5.22 and 5.32 which are those of oxymethine and unsaturated protons.

The ^{13}C NMR spectrum of compound 2, as well as that of DEPT, revealed the presence of 29 carbon atoms including 5 methyles, 10 methylenes, 10 methines and 4 quaternary carbons. This spectrum also showed a peak at δ_C 71.80

assignable to a carbon linked to a hydroxyl group, probably the C-3 carbon of sterols. At δ_C 148.62, 140.76, 137.19, 130.34, 121.69 and 109.51, were observed the presence of six double bonded carbons.

A comparison of the spectroscopic and physical data of compound 2 with those found in the literature [9-11], allowed us to attribuate to compound 2 the structure 2 which was that of Stigmasta-5,22,25-trien-3-ol [11] (Fig. 1).

Compound 3 was obtained as beige powder. It was soluble in pyridine and reacted positively to the Molish test, suggesting that compound 3 was a glycoside.

A compared analysis of the [13] C NMR spectra of compound 3 and compound 2 showed that they had almost similar signals. However, compound 3 possessed six additional carbons bonded to oxygen atoms between δ_C 62.88 and 102.60, corresponding to the sugar group identified above. In addition, the anomeric carbon appearing at δ_C 102.60 indicated that the sugar group was attached to an oxygen atom probably at position 3. This was confirmed by the strong de-shielding of the C-3 carbon in compound 3. Thus, it was obvious that compound 3 was the glucoside of compound 2.

The comparison of the NMR data of compound 3 with those from the literature [11], allowed us to identify compound 3 as Stigmasta-5,22,25-trien-3-O-β-D-glucopyranoside [11] (Fig. 1).

Compound 4 was obtained as white powder. UV λ_{max} value of compound 4 was 229 nm. The Mass spectrum (FAB-) of compound 4 showed a pseudo-molecular ion peak at m/z 680.4 $[M-H]^+$ corresponding to the molecular formula $C_{42}H_{83}NO_5$, with 2 degrees of unsaturation. The IR (KBr) spectrum of compound 4 showed absorption bands due to a hydroxyl or an amide at (3693 - 3414) cm^{-1}, a fatty acid chain at (2921 - 2851) cm^{-1} and a carbonyl group of amides at 1627 cm^{-1}.

The ^1H NMR spectrum of compound 4 showed signals assignable to an amide proton at δ_H 8.57 (1H, d, J = 8.8 Hz), two terminal methyl groups at δ_H 0.85 (6H, t, J = 6 Hz) and an oxygenated methylene at δ_H 4.50 (1H, m, H-1a) and 4.43 (1H, m, H-1b). However, we observed the appearance of three oxymethine protons at δ_H 4.62 (1H, m), 4.29 (1H, m), and 4.35 (1H, m), and a de-shielded signal at δ_H 5.12 (1H, m) that we assigned to the H-2 of sphingosine, characteristic of ceramides [12]. This spectrum also showed signals of two olefinic protons at δ_H 5.55 (1H, m) and 5.49 (1H, m) respectively, in addition to the signals of two alkylmethylenes chains, appearing as multiplets at δ_H 1.10 - 1.50, confirming that compound 4 possessed a ceramide skeleton [13].

The ^{13}C NMR spectrum of compound 4 showed signals of an amide carbonyl group (NC=O) at δ_C 175.5, one methine linked to the amide group at δ_C 52.99 and three oxymethines at δ_C 76.86 and 73.03 (overlapping). This spectrum also indicated the existence of two olefinic methine carbons respectively at δ_C 130.82 and 130.70, suggesting the presence of a double bond. A signal due to the presence of an oxymethylene group appeared at δ_C 62.04.

The position of the double bonds (C19 / C20) was identified from fragmentations observed on the electron impact mass spectrum, with the presence of fragments at m/z 57, corresponding to a butyl group and at m/z 83, corresponding to a hexenyl group. Moreover, the geometry (Trans) of the double-bond was assigned based on the chemical shifts of allylic carbons (δ_C 33.84 and 33.30). Generally, signals of carbon next to a cis double-bond appear at $\delta_C \approx 27$, while those next to trans double-bonds appear at $\delta_C \approx 32$ [14].

A comparison of spectroscopic data of compound 4 with those from the literature [15] allowed us to assign to compound 4 the structure of tithoniamide B [15] (Fig. 1).

4. Conclusion

The present phytochemical study of the dichloromethane-methanol extract of the stem bark of *Tetrorchidium didymostemon* afforded a fatty alcohol: 1-hentriacontanol, two stigmasterol derivatives: stigmasta-5,24,28-trien-3-ol and stigmasta-5,24,28-trien-3-O-β-D-glucopyranoside and one ceramide derivative: tithoniamide B. The structures of the isolated compounds were established through analysis of their spectroscopic data. These compounds are reported from *T. didymostemon* for the first time. 1-hentriacontanol has been shown to exhibit interesting antiplasmodial activity against *Plasmodium berghei* and *P. vinckei* in mice, and devoid of any toxicity [8]. This could justify the use of *T. didymostemon* in traditional medicine in the treatment of malaria.

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