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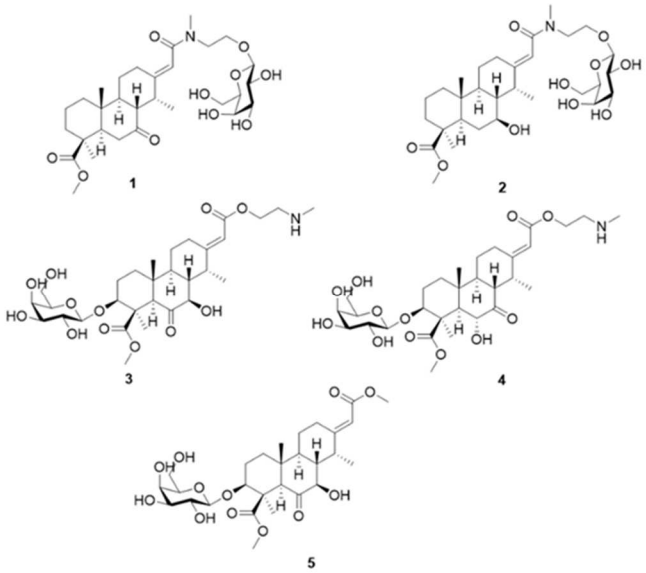
Abstract

Five new cassane-type diterpenoid heterosides, *i. e.* two cassane-type amides (**1**–**2**), two erythrophlamine-type amine esters (**3**–**4**) and a non-nitrogenous erythrophlamine analogue (**5**) were isolated from the root barks (**1**–**2**) and the seeds (**3**–**5**) of *Erythrophleum suaveolens*. Their structures were unambiguously established by interpretation of their HRESIMS, 1D and 2D NMR data, and chemical degradation for sugar determination. Compounds **3**–**5** were evaluated for their cytotoxicity against a panel of three cell lines, revealing modest to strong activities.

Erythrophleum suaveolens
[(Guill. et Perr.), Brenan]



Keywords: Fabaceae ; *Erythrophleum suaveolens* ; Diterpene ; Cassane ; Heterosides.

1. Introduction

Erythrophleum (Fabaceae-Caesalpinioideae) is a pantropical woody genus that comprises around 15 species with representatives from North-East Asia, Australia, and Africa [1]. Cassaine diterpenoid amides and amine esters, customarily referred to as cassaine alkaloids or *Erythrophleum* alkaloids were long known as the main specialized metabolites found in the genus *Erythrophleum* [2] and *Caesalpinia* [3]. The long-held interest in these metabolites is primarily related to their notorious digitalis-like action on the heart [4–7] but is also sustained to a lesser extent by their cytotoxicity against some tumor cell lines [8–10]. Among this genus, the African-native *E. suaveolens* (Guill. & Perr.) Brenan is being used in folk medicine, as a poison or an ordeal brew for people suspected of witchcraft or crimes because of its perceived toxicity. Ethnopharmacological claims are related to antiinflammatory and analgesic effects so that this plant steps in the treatment of various ailments such as oedema, rheumatism, and asthma [11]. This plant is also endowed with antibacterial activities [12] and the wood was also reported to exert anti-termites and anti-fungal properties [13]. Former phytochemical investigations on this species resulted in the isolation of a wealth of cassane-type diterpenoids [14,15], with new molecules continuously being reported from this plant source [16–19]. In the frame of our continuous efforts dedicated to the description of new *Erythrophleum* alkaloids [16–18], we herein report the isolation and structure elucidation of 5 novel cassane derivatives from the seeds and root bark of *Erythrophleum suaveolens*. The compounds have been determined, and their spectroscopic data fully assigned, based on an extensive set of NMR sequences and on chemical degradation. Compounds available in sufficient amount (**3**-**5**) were also evaluated for their cytotoxicity against a panel of 5 cell lines.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured at 25°C on a Polaar 32 Polarimeter. IR spectra were recorded with a Bruker Vector 22. The NMR spectra were acquired on Bruker AM-300 (300 MHz), AM-400 (400 MHz), and AM-500 MHz using CD_3OD and D_2O as solvents. Solvent residual signal was used for calibration. A Sunfire® preparative C_{18} column (150 × 19 mm, i. d. 5 μm, Waters) was used for preparative HPLC separation using a Waters Delta Prep fitted with a binary pump (Waters 2525) and a photodiode array detector (190-600 nm, Waters 2996). A silica 24 g Grace cartridge was used for flash chromatography using an Armen Instrument spot liquid chromatography flash device.

Open column chromatographies were carried out on silica gel 60 (40–63 µm) or with Sephadex® LH-20 (25–100 µm) (Pharmacia Fine Chemicals, USA). TLC analyses were carried out on precoated silica gel 60 F_{254} (Merck, Darmstadt, Germany), and spots were visualized by spraying the plates with Dragendorff reagent, 10% H_2SO_4 solution or phosphomolybdic acid followed by heating.

2.2. Plant material

Seeds and root bark of *Erythrophleum suaveolens* (Guill. & Perr.) Brenan were collected in Toumodi province (Côte d'Ivoire) in December 2014 and authenticated by Pr. Ipou Ipou Joseph. Voucher specimens (n° 10 KABLAN ES-2014 and n° DIBI ES-2014) were deposited in the herbarium of Centre National de Floristique (CNF), Université Felix Houphouet Boigny, Abidjan (Côte d'Ivoire).

2.3. Extraction and Isolation

The air-dried powdered root bark (700 g) was extracted three times with methanol (7 L, 24 h) at room temperature. The extracts were combined and evaporated to dryness (32.3 g) under reduced pressure. A 15 g aliquot of this extract was mixed with a mixture of MeOH and CH_2Cl_2 (1:1; 50 mL),

alkalinized with a few drops of 6M NH_4OH. This solution was extracted with a solution of 1M sulfuric acid (50 mL). The acid/aqueous phase was then extracted twice with CH_2Cl_2 (100 mL each). The remnant aqueous phase was alkalinized using 6M NH_4OH (pH 10) and extracted with EtOAc (4 x 100 mL) to yield 2 g of ethyl acetate extract. The extract was subjected to flash chromatography using a silica 24 g Grace cartridge with a gradient of CH_2Cl_2-MeOH (1:0 to 0:1) at 24 mL/min to afford seven fractions (F1-7), according to their TLC profiles. Fraction F4 (104 mg) was further fractionated on a Sephadex® LH-20 column using an isocratic regime of CH_2Cl_2/MeOH (1:1), that resulted in three subfractions (F41-F43). Fraction F42 (32 mg) was selected and taken through preparative HPLC separation using a gradient of CH_3CN-H_2O with 0.1% formic acid (85:15 to 50:50) to afford **1** (2.4 mg). Fraction F5 (132 mg) was fractionated on a column of Sephadex® LH-20 using CH_2Cl_2/MeOH (1:1) mobile phase to give 5 fractions (F51-F55). Further preparative HPLC fractionation of subfraction F55 (32 mg) yielded **2** (2.7 mg).

The air-dried seeds (10 g) were milled prior to being macerated three times with methanol (100 mL, 24 h) at ambient temperature. The filtrated was concentrated in vacuum to afford a crude extract (1.4 g). This residue was subjected to preparative HPLC using a 15 min gradient of CH_3CN-H_2O (95:5 to 0:100) to afford **3** (57.6 mg), **4** (13.6 mg), and **5** (13.2 mg).

2.3.1. *22-O-β-D-galactopyranosyl-norcassamide* (**1**)

White amorphous solid; $[\alpha]^{20}_D$ −132.1 (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 229 (4.17) nm; IR ν_{max} (cm^{-1}) 3395, 2925, 1775, 1787, 1721, 1579, 1527, 1460, 1242, 1150, 969, 842, 663 cm^{-1}; HRESIMS *m/z* 582.3245 $[M + H]^+$ (calcd for $C_{30}H_{48}NO_{10}$, 582.3272). For ^1H and ^{13}C NMR (CD_3OD) spectroscopic data, see Tables 1 and 2.

2.3.2. *22-O-β-D-galactopyranosyl-norcassamidide* (**2**)

White amorphous solid; $[\alpha]^{20}_D$ -160.1 (*c* 0.05, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 230 (4.15) nm; IR ν_{max} (cm^{-1}) 3441, 2936, 1716, 1695, 1589, 1159, 1079, 749, 664; HRESIMS *m/z* 584.3417 $[M + H]^+$ (calcd for $C_{30}H_{50}NO_{10}$, 584.3429). For ^1H and ^{13}C NMR (CD_3OD) spectroscopic data, see Tables 1 and 2.

2.3.3. *3-O-β-D-galactopyranosyl-norerythrosuamine* (**3**)

White amorphous solid; $[\alpha]^{20}_D$ -66.1 (*c* 0.272, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.17) nm; IR ν_{max} (cm^{-1}) 3395, 3020, 2947, 2832, 1727, 1723, 1648, 1595, 1471, 1280, 1181, 1024, 1009, 987, 872, 756 cm^{-1}; HRESIMS *m/z* 614.3173 $[M + H]^+$ (calcd for $C_{30}H_{48}NO_{12}$, 614.3171). For ^1H and ^{13}C NMR (CD_3OD) spectroscopic data, see Tables 1 and 2.

2.3.4. *3-O-β-D-galactopyranosyl-6α-hydroxynorcassamine* (**4**)

White amorphous solid; $[\alpha]^{20}_D$ -66.4 (*c* 0.106, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.18) nm; IR ν_{max} (cm^{-1}) 3018, 1718, 1648, 1451, 1216, 1154, 1078, 929, 745; HRESIMS *m/z* 614.3185 $[M + H]^+$ (calcd for $C_{30}H_{48}NO_{12}$, 614.3171). For ^1H and ^{13}C NMR (CD_3OD) spectroscopic data, see Tables 1 and 2.

2.3.5. *3-O-β-D-galactopyranosyl-erythrosuamic acid methyl ester* (**5**)

White amorphous solid; $[\alpha]^{20}_D$ -42.0 (*c* 0.142, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.17) nm; IR ν_{max} (cm^{-1}) 3020, 2951, 1717, 1649, 1595, 1460, 1391, 1260, 1215, 1173, 1076, 1038, 988, 941, 873, 745, 667; HRESIMS *m/z* 593.2210 $[M + Na]^+$ (calcd for $C_{27}H_{38}O_{13}Na$, 593.2205). For ^1H and ^{13}C NMR (CD_3OD) spectroscopic data, see Tables 1 and 2.

2.4. Acid hydrolysis for sugar determination

NMR tube degradation method was performed to determine the sugar as formerly reported [20]. Accordingly, compounds **1** and **2** (1 mg each) were dissolved in 0.5 mL of D_2SO_4 and the hydrolysis proceeded during 4 h at 37°C. The 1H NMR spectra were then acquired with test conditions as described in Giner et al [20].

2.5. Cytotoxicity assays

Different cancer cell lines were obtained from the American type Culture Collection (ATCC, Rockville, MD). Cancer cell lines were cultured according to the supplier's instructions. Human HCT-116 colorectal carcinoma and SK-OV-3 ovary carcinoma were grown in Gibco McCoy's 5A supplemented with 10% fetal calf serum (FCS) and 1% glutamine. MCF7 and A549 cells were cultured in RPMI 1640 supplemented with 10% Fetal Bovine Serum and 1% glutamine. Cell lines were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Cell viability was determined by a luminescent assay according to the manufacturer's instructions (Promega, Madison, WI, USA). For IC_{50} determination, the cells were seeded in 96-well plates (3×10^3 cells/well) containing 100 µL of growth medium. After 24 h of culture, the cells were treated with the test compounds at 10 different concentrations, ranging from 0.01 to 10 µM. Control cells were treated with the vehicle. Experiments were performed in triplicate. After 72 h of incubation, 100 µL of CellTiter Glo Reagent was added for 15 min before recording luminescence with a spectrophotometric plate reader PolarStar Omega (BMG LabTech). Dose-response curves were plotted with Graph Prism software and the IC_{50} values were calculated using the Graph Prism software from polynomial curves (four or five-parameter logistic equations). Doxorubicin was used as a positive control.

3. Results and Discussion

Four new *Erythrophleum* alkaloids (**1**–**4**) and a new non-nitrogenous erythrophlamine analogue, **5** were isolated from both the seeds and root bark of *Erythrophleum suaveolens* in this study.

The molecular formula of **1** was established as $C_{30}H_{47}NO_{10}$ based on ^{13}C NMR and HRESIMS data. The HRESIMS peak at *m/z* 582.3245 (calcd for $C_{30}H_{48}NO_{10}$, 582.3272) suggests the presence of 8 degrees of unsaturation. The IR spectrum revealed bands at 3395 and 1721 cm^{-1} diagnostic of the presence of hydroxyl and carbonyl groups in the molecule. The ^{13}C NMR spectrum revealed 30 carbon signals, 24 of which were assigned to a diterpenoid core and 6 to a monosaccharide unit. This inference was backed up by the occurrence of a fragment at *m/z* 420.2708 $[(M+H)-162]^+$ that hinted either the loss of a hexose without the glycosidic oxygen or the loss of a deoxyhexose unit with the glycosidic oxygen [21]. The ^{13}C NMR spectrum, in conjunction with HSQC, displayed resonances for 3 carbonyls (δ_C 212.8, 178.9, 171.8), two olefinic carbons (δ_C 155.6, 116.4) and one *N*-methyl (δ_C 37.8), with a chemical shift diagnostic of an amide bond [22]. Resonances for three methyls (δ_H 1.17, 1.06, 0.84), one methoxy (δ_H 3.69), one exocyclic olefinic proton (δ_H 5.87) and two heteroatom-bonded methylenic protons (δ_H 3.88/3.60 and 3.35) were detected in the ^1H NMR spectrum. These

spectroscopic features were indicative of a cassaine-type diterpenoic acid, which is amide bonded to a *N*-methylaminoethanol residue. This is further supported by the cautious examination of HMBC cross-peaks, as outlined in Fig. 2. A cursory examination of the ^{1}H NMR data of **1** revealed that the signal of H-15 was split into two different signals of nearly equal intensities (Table 1). The presence of the N-Me group probably restricts the rotation of the amide C-N bond to result in two distinct conformers [23]. Although undiscussed, such spectral features can be observed in the ^{1}H NMR spectrum of formerly reported cassane diterpenoid amides [16]. Other signals could have been expected to split in a similar manner as a consequence of these two conformations. These differences were probably too subtle to be observed in the crowded, highly-overlapping NMR data of **1**. The magnitudes of the vicinal coupling constants of H-5 (dd, *J* = 14.1, 3.4 Hz) were indicative of its being α-disposed, while the signal at δ_H 1.58 tended to indicate a 7-keto moiety [24], as subsequently confirmed by HMBC correlations. The *E*-geometry of the olefinic bond $\Delta^{13(15)}$ was determined based on the NOE crosspeak between H-14 (δ_H 2.98) and H-15 (δ_H 5.87). This is in line with all formerly reported *Erythrophleum* alkaloids [7]. The *trans*/*anti*/*trans* configuration of all cassane-type diterpenes reported to date straightforwardly determines the *β* -orientation of the protons at C-8 and the *α*-orientation of both the protons H-5 and H-9 [25,26], this latter point being supported by the H-5/H-9 NOE crosspeak. From there, the key NOE crosspeaks between the methine proton at δ_H 1.70 (H-9) and the methyl protons at δ_H 1.06 (H_3-17) and between the methine proton at δ_H 1.58 (H-5) and the methyl protons at δ_H 1.17 (H_3-18) indicated that these protons were *α*-oriented. Conversely, the NOE correlations between the methine proton at δ_H 2.35 (H-8) and both the methyl protons at δ_H 0.84 (H_3-20) and the methine proton at δ_H 2.98 (H-14) established their *β*-disposition. The ^{13}C NMR spectroscopic data were fully consistent with this configuration [27], which is very classical among tricyclic cassanes [3,28]. The genine part of the molecule was thus unambiguously determined as norcassamide [24], with the sole difference being the chemical shift of C-22 at 7 ppm downfield in **1** compared to the former. This chemical shift value led to surmise that **1** was a monodesmosic glycoside with a monosaccharide unit linked to this carbon, as validated by the HMBC cross-peak between the anomeric proton at δ_H 4.26 and C-22 (δ_C 68.1). The nature of the sugar was determined based on the ^{1}H NMR analysis of the monosaccharide following its acid hydrolysis (S10, supporting information). The chemical shifts, coupling constants and integral ratios of the anomeric proton signals were indeed proved to be diagnostic of a given sugar [20]. Accordingly, the doublets at δ_H 5.30 (*J* = 3.8 Hz) and 4.62 (*J* = 7.9 Hz) were diagnostic of a galactose moiety. These spectroscopic data defined the structure of **1**, namely 22-*O*-*β*-D-galactopyranosyl-norcassamide, as shown in Fig. 1.

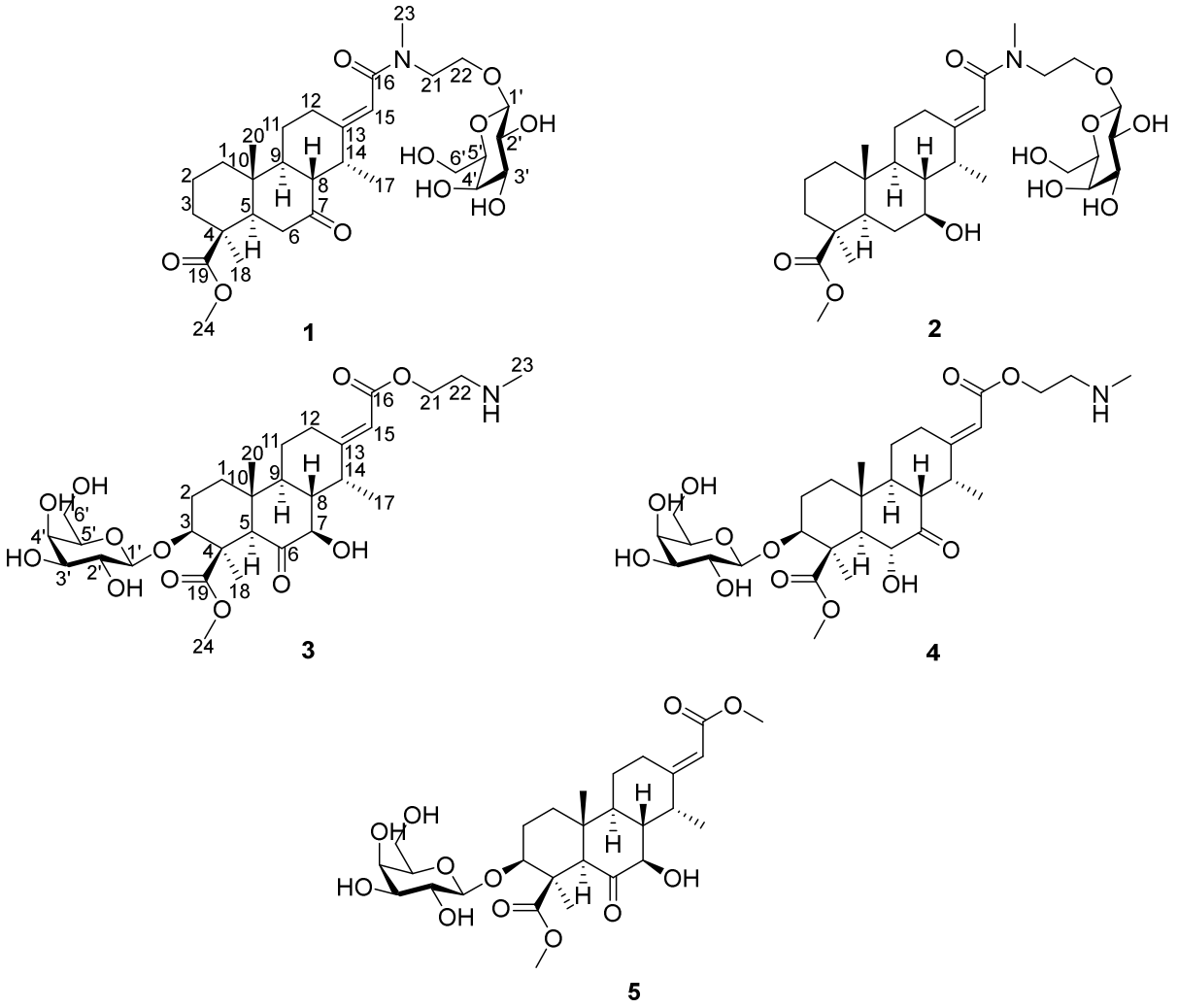


Figure 1. Chemical structures of **1**–**5**

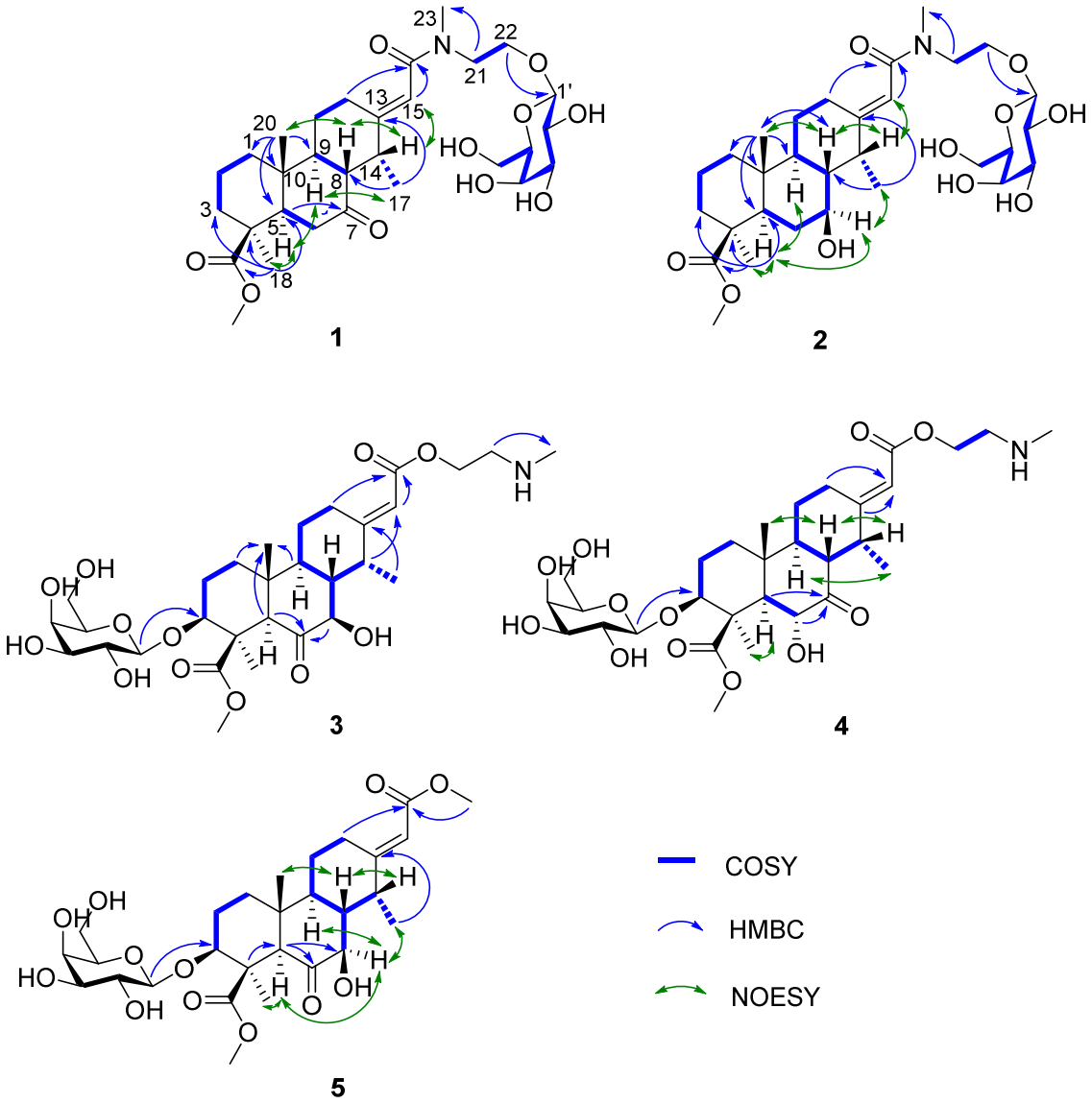


Figure 2. Key COSY, HMBC and NOESY correlations of **1**–**5**

Compound **2**, isolated as a white amorphous solid, gave a molecular formula of $C_{30}H_{49}NO_{10}$ based on its positive-ion HRESIMS data at *m/z* 584.3417 (calcd for $C_{30}H_{50}NO_{10}$, 584.3429), differing from **1** by two supplementary hydrogen atoms. Both the ^{1}H and ^{13}C NMR data were similar to those of **1**, with the sole difference being concentrated around C-7 which was upfield shifted to δ_C 72.2 while the neighbouring positions were 6 ppm downfield in **2** compared to **1**. These spectroscopic features indicated the replacement of the carbonyl group by a hydroxy moiety, as validated by COSY and HMBC correlations outlined in Figure 2. The elevated coupling constant of H-7 was diagnostic of its axial disposition and hence of a *β*-equatorial hydroxy group [29]. Full assignment of the ^{1}H and ^{13}C NMR chemical shifts (Tables 1 and 2) based on the thorough analysis of 2D NMR sequences revealed that the rest of the scaffold displays the canonical substitution pattern and stereochemistry of cassanes. This identified the genine component of **2** as norcassamidide, which – as far as can be ascertained – lacked fully assigned spectroscopic data [30–32]. The key NOE correlations from H-8 to both H_3-20 and H-14 indicated their *β*-orientation. Conversely, the NOE crosspeaks between H-7 and both H-5 and H_3-17 determined their *α*-disposition. Chemical shifts, coupling constants, and integral ratios following acid hydrolysis also determined the sugar unit as a galactose moiety, establishing the structure of **2**, 22-*O*-*β*-D-galactopyranosyl-norcassamidide, as indicated in Fig. 1.

Compound **3** was obtained as a white amorphous solid. Its molecular formula, $C_{30}H_{47}NO_{12}$, was established from the prominent protonated molecular ion peak at *m/z* 614.3173 (calcd for $C_{30}H_{48}NO_{12}$, 614.3171) in HRESIMS data. It differs from **2** by the loss of two hydrogen atoms and by the gain of two additional oxygen atoms. Despite important similarities between NMR data of **3** with those of **1** and **2**, some salient differences was however noted. At first, the *N*-methyl group was upfield shifted to a value of δ_C 33.8. This with the upfield shift shown by C-16, was indicative of an ester amine side chain, rather than an amide [32,33], defining either a cassamine or an erythrophlamine analogue. Likewise, the chemical shifts of C-21 and C-22 were in line with this inference [22,33], as further supported by the HMBC correlations outlined in Fig. 2. Evidence for a 6-keto-7-*β*-hydroxy grouping was provided by the H-7*β* signal that occurred as a doublet (J = 12 Hz) at δ_H 4.03 and the H-5 singlet at δ_H 2.64 [34,35]. The downfield chemical shift at C-3 resonating at δ_C 88.4 was evocative of a glycosidic shift [36], which was proved by the HMBC correlation from the anomeric proton at δ_H 4.34 to this carbon. Its 3*β*-orientation could be deduced from the magnitude of H-3 oxymethine coupling constant [24]. Again, the NOESY correlations determined a similar configuration for **3**. The ^{1}H and ^{13}C NMR spectra of the sugar residue were superimposable to those of **1** and **2** and were in excellent agreement with former literature reports for galactose [37,38]. Altogether, these spectrosopic features determined the structure of **3** as 3-*O*-*β*-D-galactopyranosyl-norerythrosuamine (Fig. 1). Noteworthily, a closely similar metabolite, comprising the same genine with β-D-glucopyranose instead of β-D-galactose had been reported from *Erythrophleum lasianthum* by Verotta and co-workers [33].

Compound **4**, isolated as a white amorphous solid, gave the molecular formula $C_{30}H_{47}NO_{12}$, as deduced by the protonated molecular ion peak at *m/z* 614.3185 in the HR-ESI mass spectrum, making it an isomer of **3**. Overall, the ^{1}H and ^{13}C NMR data of **4** were almost similar to those of **3**. The signals related to the methine proton H-5 [δ_H 1.51 (d, J = 11.5 Hz)] and H-6*β* [δ_H 4.31 (d, J = 11.5 Hz)] were the most notable differences there between. These spectroscopic data were similar to those of erythrophleguine, determining a 6*α*-hydroxy-7-keto group [39]. Consistently, the NOE correlations between H_3-17 and H-9 and between H-5 and H_3-18 determined the *α*-orientation of these protons, while the NOE crosspeaks of H-8 with both H-14 and H_3-20 defined the *β*-disposition of all these protons. The detailed comparison of ^{1}H and ^{13}C NMR data for compounds **3** and **4** established that the nature and anomeric configuration of the monosaccharide were identical, determining **4** as 3-*O*-*β*-D-galactopyranosyl-6α-hydroxynorcassamine.

Compound **5** was isolated as a colourless amorphous solid. Its molecular formula $C_{27}H_{38}O_{13}$, as deduced by the sodiated molecular ion peak at *m/z* 593.2210, indicated a non-nitrogenous cassane. It differs from **3** and **4** by the absence of a $–CH_2NHCH_3$ unit. The thorough analysis of 1D and 2D NMR data of **5** revealed it to be a structural analogue of **3** with an identical tricyclic scaffold and a shortened side chain that indeed lacked the usual *N*-methylaminoethanol moiety. Compound **5** displayed a terminal methyl ester group, as evidenced by the HMBC correlations outlined in Fig. 2. Such side chains were formerly reported to occur within cassane diterpenes [17,40,41]. The identical sugar-related NMR signals established **5** as 3-*O*-*β*-D-galactopyranosyl-depicted in Figure 1, and we named it 3-O-β-D-galactopyranosyl erythrosuamic acid. Here as well, the NOESY spectrum provided a valuable support in determining the typical stereochemistry of a cassane-type diterpene. At first, the NOE crosspeaks between H-7 and H_3-17/H-9 and H-5; and between H-5 and H_3-18 established the *α*-orientation of these protons. Then, the NOE correlations between H-8 and both H_3-20 and H-14 allowed these protons to be *β*-oriented. The structural assignment of **5** based on its NMR data is fully supported by comparison to the data of **3**.

Table 1. ^{1}H NMR Spectroscopic Data of Compounds 1–5 (δ in ppm, J in Hz) (CD_3OD, 400 MHz).

no.	**1**	**2**	**3**	**4**	**5**
1	1.41 (1H, m) 1.88 (1H, m)	1.47 (1H, m) 1.85 (1H, m)	1.42 (1H, m) 1.82 (1H, m)	1.77 (1H, m) 1.25 (1H, m)	1.46 (1H, m) 1.83 (1H, m)
2	1.51 (1H, m) 1.88 (1H, m)	1.44 (1H, m) 1.82 (1H, m)	2.25 (1H, m) 1.22 (1H, m)	2.25 (1H, m) 1.16 (1H, m)	2.25 (1H, m) 1.16 (1H, m)
3	1.16 (1H, m) 2.23 (1H, m)	1.09 (1H, m) 2.15 (1H, m)	3.49 (1H, dd, 12.0, 4.0)	3.31 (1H, dd, 12.0, 4.0)	3.54 (1H, dd, 12.0, 4.0)
4	-	-	-	-	-
5	1.58 (1H, dd, 14.1, 3.4)	1.15 (1H, m)	2.64 (1H, s)	1.51 (1H, d, 11.5)	2.69 (1H, s)
6	2.62 (1H, m) 2.89 (1H, m)	1.75 (1H, m) 2.15 (1H, m)	-	4.31 (1H, d, 11.5)	-
7	-	3.40 (1H, dd, 10.7, 3.9)	4.03 (1H, d, 12.0)	-	4.06 (1H, d, 12.0)
8	2.35 (1H, d, 12.5)	1.49 (1H, m)	1.86 (1H, m)	2.43 (1H, dd, 12.0, 4.0)	1.89 (1H, m)
9	1.70 (1H, m)	1.72 (1H, m)	1.77 (1H, m)	1.73 (1H, dd)	1.84 (1H, m)
10	-	-	-	-	-
11	1.28 (1H, m) 1.92 (1H, m)	1.07 (1H, m) 1.82 (1H, m)	1.15 (1H, m) 1.92 (1H, m)	1.16 (1H, m) 2.17 (1H, m)	1.12 (1H, m) 2.04 (1H, m)
12	2.07 (1H, m) 2.77 (1H, m)	2.00 (1H, m) 2.73 (1H, m)	3.76 (1H, m) 2.07 (1H, m)	3.77 (1H, m) 2.06 (1H, m)	3.70 (1H, m) 2.24 (1H, m)
13	-	-	-	-	-
14	2.98 (1H, m)	2.84 (1H, m)	2.79 (1H, m)	2.97 (1H, m)	2.80 (1H, s)
15	5.87 (0.5 H, s)[a] 6.03 (0.5 H, s)[b]	5.83 (0.6 H, s)[a] 5.99 (0.4 H, s)[b]	5.78 (1H, s)	5.78 (1H, s)	5.74 (1H, s)
16	-	-	-	-	-
17	1.06 (3H, d, 6.8)	1.09 (3H, d, 6.8)	1.19 (3H, d, 8.0)	1.09 (3H, d, 8.0)	1.23 (3H, d, 8.0)
18	1.17 (3H, s)	1.20 (3H, s)	1.33 (3H, s)	1.31 (3H, s)	1.38 (3H, s)
19	-	-	-	-	-
20	0.84 (3H, s)	0.80 (3H, s)	0.90 (3H, s)	1.05 (3H, s)	0.97 (3H, s)
21	3.55 (2H, m)	3.51 (2H, m)	4.34 (2H, m)	4.31 (2H, m)	3.69 (3H, s)
22	3.75 (2H, m)	3.75 (2H, m)	3.32 (2H, m)	3.31 (2H, m)	3.70 (3H, s)
23	3.10 (3H, s)	3.12 (3H, s)	2.71 (3H, s)	2.70 (3H, s)	-
24	3.66 (3H, s)	3.63 (3H, s)	3.65 (3H, s)	3.65 (3H, s)	-
1′	4.26 (1H, d, 7.7)	4.26 (1H, d, 7.7)	4.34 (1H, d, 7.7)	4.31 (1H, d, 7.7)	4.38 (1H, d, 7.7)
2′	3.15 (1H, d, 9.7, 7.7)	3.15 (1H, d, 9.7, 7.7)	3.11 (1H, d, 9.7, 7.7)	3.23 (1H, d, 9.7, 7.7)	3.17 (1H, d, 9.7, 7.7)
3′	3.25 (1H, dd, 9.7, 3.5)	3.26 (1H, dd, 9.7, 3.5)	3.24 (1H, dd, 9.7, 3.5)	3.25 (1H, dd, 9.7, 3.5)	3.29 (1H, dd, 9.7, 3.5)
4′	3.26 (1H, dd, 4.5, 2.5)	3.27 (1H, dd, 4.5, 2.5)	3.34 (1H, dd, 4.0, 2.5)	3.31 (1H, dd, 4.0, 2.5)	3.30 (1H, dd, 4.0, 2.5)
5′	3.35 (1H, m)	3.37 (1H, m)	3.29 (1H, m)	3.29 (1H, m)	3.36 (1H, m)
6′	3.60 (1H, dd, 11.8, 2.0) 3.88 (1H, dd, 11.8, 5.4)	3.67 (1H, dd, 11.8, 2.0) 3.88 (1H, dd, 11.8, 5.4)	3.56 (1H, dd, 11.8, 2.0) 3.83 (1H, dd, 11.8, 5.4)	3.65 (1H, dd, 11.8, 2.0) 3.83 (1H, dd, 11.8, 5.4)	3.69 (1H, dd, 11.8, 2.0) 3.87 (1H, dd, 11.8, 5.4)

[a] Chemical shift data of rotamer 1 [b] Chemical shift data of rotamer 2

Table 2. ^{13}C NMR Spectroscopic Data of Compounds 1–5 (δ in ppm) (CD_3OD, 100 MHz).

no.	**1**	**2**	**3**	**4**	**5**
1	39.7	40.5	37.5	38.1	37.6
2	20.5	20.5	24.8	25.0	24.8
3	38.6	38.9	88.4	89.2	88.9
4	45.2	45.2	49.2	49.0	49.0
5	54.7	54.0	65.3	59.0	65.4
6	40.8	34.1	209.8	76.3	209.8

7	212.8	72.2	77.0	210.7	77.1
8	54.6	48.5	52.1	53.3	52.1
9	47.9	46.8	47.3	48.0	47.4
10	38.0	38.1	43.1	38.3	43.2
11	28.2	27.7	27.6	28.5	27.7
12	26.0	26.3	27.2	27.4	27.3
13	155.8	157.7	167.3	167.3	167.8
14	41.1	40.1	41.8	41.1	41.8
15	116.4	115.8	113.1	113.1	113.9
16	171.8	171.8	169.7	168.9	168.8
17	14.8	14.0	14.0	14.2	14.0
18	27.9	29.1	26.3	27.7	26.3
19	178.9	179.2	175.7	176.5	175.7
20	12.2	13.4	15.0	15.4	15.0
21	53.7	53.7	60.0	60.0	51.4
22	68.2	68.1	48.2	48.7	51.8
23	37.8	37.8	33.8	33.8	-
24	51.7	51.7	52.0	51.9	-
1′	104.6	104.5	106.5	107.2	106.5
2′	75.1	75.1	75.5	75.5	75.5
3′	71.8	71.7	71.5	71.5	71.5
4′	71.5	71.5	71.4	71.4	71.5
5′	78.1	78.1	78.0	78.0	78.1
6′	62.9	62.9	62.7	62.8	62.7

Cytotoxicity of the compounds

Owing to a shortage of material, only compounds **3**, **4**, and **5** could be evaluated for their cytotoxicity. Compound **3** was generally toxic against all the cells tested. Compound **4** was active against 2 cell lines (A549 and HCT116) while compound **5** was inactive against all the selected cell lines (Table 3). These results complete former studies having outlined the cytotoxic activities of cassane-type diterpenes [3,7].

Table 3. Cytotoxicity against cultured cell lines (IC_{50}) (μM)

Tested compound	A549	MCF7	HCT116
3	0.50±0.01	7.30±0.32	6.26±0.24
4	4.92±0.48	> 10	6.75±0.09
5	> 10	> 10	> 10
Doxorubicin (nM)	56.0 ± 0.8	120.0 ± 9.0	90.0 ± 3.0

Each value represents the mean of triplicate studies.

4. Conclusions

Five new diterpenoids identified as *22-O-β-D-galactopyranosyl-norcassamide* (**1**), *22-O-β-D-galactopyranosyl-norcassamidide* (**2**), *3-O-β-D-galactopyranosyl-norerythrosuamine* (**3**), *3-O-β-D-galactopyranosyl-6α-hydroxynorcassamine* (**4**) and *3-O-β-D-galactopyranosyl erythrosuamic acid* (**5**) were isolated from the bark (**1**–**2**) and seeds (**3**–**5**) of *Erythrophleum suaveolens*. Compounds **3**, **4** and **5** demonstrated varied levels of cytotoxic activity against selected cell lines.

Declaration of Competing Interest

The authors declare no conflict of interest for this study.

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Appendix A. Supplementary data.

1D, 2D NMR, HRESIMS and IR spectra of compounds **1**-**5** are available as Supplementary data. Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote...

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