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Cameroonemide A: a new ceramide from *Helichrysum cameroonense*

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NOTE

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From the extracts of all parts of the plant *Helichrysum cameroonense*, five compounds were isolated and identified. One of them, a ceramide, named cameroonemide A (**1**), is reported for the first time as a new natural product. Its structure was determined by comprehensive analyses of their 1D and 2D NMR and HR-EI-MS spectral data. The remaining four known compounds were identified by comparing their spectroscopic data with those reported in the literature as kaurenoic acid (**2**), 3-acetyloxykaurenoic acid (**3**), β-sitosterol (**4**), and β-sitosterol glucopyranoside (**5**). Preliminary studies showed that 3-acetyloxykaurenoic acid (**3**) inhibited the alga *Chlorella fusca*, while kaurenoic acid (**2**) showed strong antibacterial activity against *Bacillus megaterium*.

Keywords: *Helichrysum cameroonense*; Asteraceae; ceramide; antimicrobial activity

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1. Introduction

Helichrysum species produce various secondary metabolites (acetophenones, flavonoids, and phloroglucinols) as a biochemical defense mechanism (chemical barrier) against bacteria and fungi [1]. This is of interest, since the chemical diversity of the metabolites produced indicates the use of different metabolic pathways in this defense mechanism. As part of an ongoing program to investigate the medicinal potential of Mount Cameroon savannah plants, we examined *Helichrysum cameroonense* Hutch. & Dalziel for possible biologically active metabolites. *H. cameroonense* Hutch. & Dalziel belongs to Inulae (Family Asteraceae), a small tribe of 13 genera and 37 species, which occur in Cameroon and some other parts of the world [2]. *H. cameroonense* Hutch. & Dalziel is found at an altitude of 1500 m in the western savannah of Cameroon, and is commonly called 'strawflower' [2]. This plant has no recorded description of medicinal use or chemical characterization. However, other members of the genus *Helichrysum* have several important medicinal applications, e.g. the Southern Sotho inhale the smoke of *H. caespititium* for relief of head and chest colds, and it is also used as a dressing for open wounds during circumcision rites [1]. In Zululand, the smoke of the burning plant material of *H. decorum* is inhaled by diviners to induce trances [3]. Chemical investigation of *H. cameroonense* Hutch. & Dalziel led to the isolation of one new compound, a ceramide named cameroo-

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nemide A (**1**). In addition, four known compounds, including two kaurane-type diterpenoids, kaurenoic acid (**2**) and 3-acetyloxykaurenoic acid (**3**), and two steroids, β-sitosterol (**4**) and β-sitosterol glucopyranoside (**5**), were isolated from *H. cameroonense* (Figure 1).

2. Results and discussion

The dried and powdered whole plants of *H. cameroonense* were extracted with MeOH–CH_2Cl_2. The residue obtained after evaporation of the solvent was fractionated between *n*-hexane and water, followed by conventional purification procedures of the *n*-hexane extract and silica gel column chromatography (CC), resulting in the isolation of five constituents, including one new ceramide (**1**) and four known compounds (**2**–**5**).

Compound **1** was isolated as an amorphous powder. The molecular formula was determined to be $C_{43}H_{85}NO_5$ by HR-EI-MS. The IR spectrum showed absorption bands at 3600 (hydroxyl), 3434, 1656, 1510 (amide), 2930, 2850, and 1465 (aliphatic) cm^{-1}, suggesting that it is a fatty acid amide [4–14]. The ^{1}H NMR spectrum (in $CDCl_3 + CD_3OD$, see Section 3) showed signals from two terminal methyl groups [δ 0.70 (6H, H-22′, 21)], aliphatic methylenes [δ 1.20–1.25], a methylene group [δ 3.50 (1H, H-1b), 3.60 (1H, H-1a)], four methine groups [δ 3.30 (1H, H-4), 3.35 (1H, H-3), 3.79 (1H, H-2′), 3.91 (1H, H-2)], disubstituted olefinic protons [δ 5.11 (m, 1H, H-13), 5.16 (m, 1H, H-12)], and an amide proton [δ 7.35 (1H)] [4–14]. The ^{13}C NMR spectrum (see Section 3) showed characteristic signals due to an amide carbonyl at δ 175.7 and a methine carbon linked to amide nitrogen at δ 53.5. These spectral data and the molecular formula suggested that compound **1** was a ceramide [4–14]. The *trans* (*E*) configuration of the double bond was evidenced by the chemical shifts of the carbons next to the double bond at δ 32.3 (C-11) and 32.1 (C-14) in **1** [15]. The chemical shifts for *cis* (*Z*) double bonds are usually in the range of δ 27–28 [4,5,15,16].

The length of the fatty acid chain was determined by EI-MS, which showed significant fragment ion peaks at *m/z* 339 $[CH_3(CH_2)_{19}CH(OH)CO]^+$, 354 $[CH_3(CH_2)_{19}CH(OH)CONH]^+$, and 411 $[CH_3(CH_2)_{19}CH(OH)C(OH){=}NC({=}CH_2)CH_2OH]^+$. The length of the long chain base

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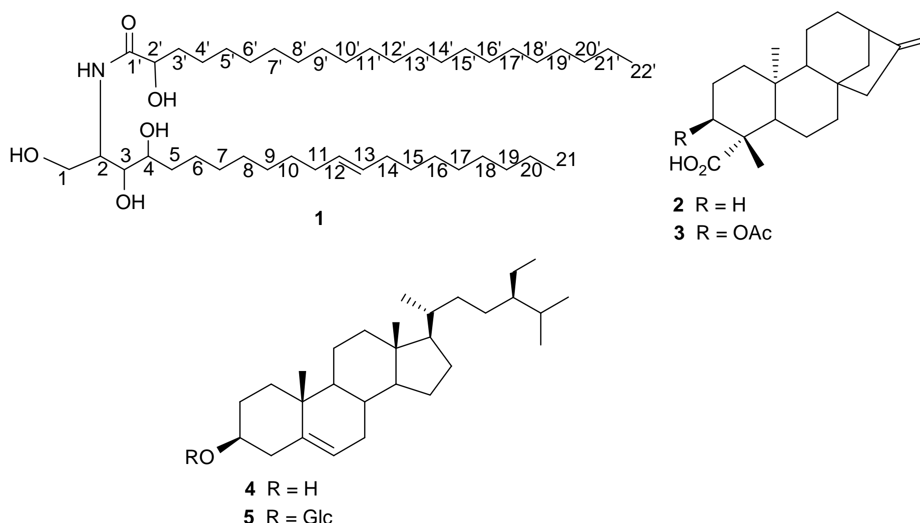

Figure 1. Structures of compounds **1**–**5** isolated from *H. cameroonense*.

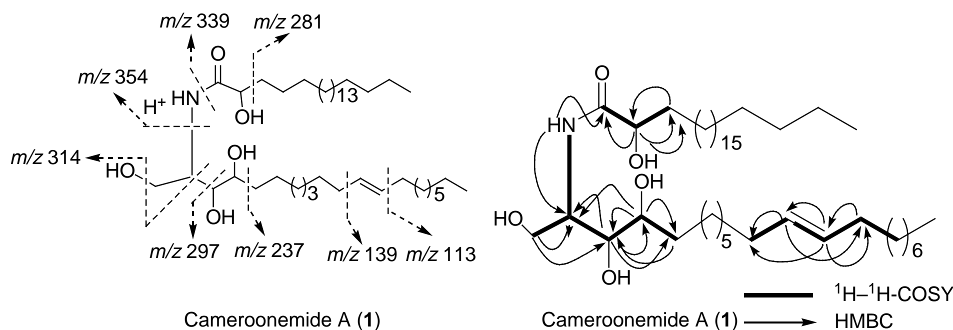

Figure 2. Key fragment ions and important $^1H-^1H$-COSY and HMBC correlations for cameroonemide A (**1**).

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was determined by the characteristic ions at *m/z* 398 $[M-CH_3(CH_2)_{14}(CH)_2(CHOH)_2]^+$, 297 $[CH_3(CH_2)_{14}(CH)_2(CHOH)_2]^+$, and 314 $[CH_3(CH_2)_{14}(CH)_2(CHOH)_2OH]^+$ in the EI-MS [4–14]. This also confirmed the position of the double bond in the long chain base. The typical fragment ion at *m/z* 597 was formed by the elimination of heptene from $[M]^+$ through the McLafferty rearrangement [11,17].

Detailed analysis of the $^1H-^1H$ COSY spectrum of **1** implied connectivities for an amide proton (δ 7.35) to H-2; H-2 to H-1a, H-1b, and H-3; and H-3 to H-2 and H-4 (Figure 2). No cross peaks of the signal were observed at δ 3.79 with any downfield proton signals, but interpretation of the HMBC spectrum revealed that this proton signal showed strong correlation with C-1′ (δ 175.5). This suggested that the fourth hydroxyl group is present at C-2′ of the fatty acid chain.

The positions of the three hydroxyl groups in the long chain base were further confirmed from the mass fragmentation pattern (Figure 2) as well as from the HMBC correlations (Figure 2). Thus, the long chain base and fatty acid of **1** must be 2-amino-12-henicosene-1,3,4-triol and 2-hydroxydocosanoic acid, respectively. On the basis of this evidence, the structure of **1** was determined to be 1,3,4-trihydroxy-2-docosanoyl-amino-12*E*-henicosene. The configuration at the chiral centers of C-2, C-2′, C-3, and C-4 could not be established from these spectral data. We have named the compound cameroonemide A after the producing organism, *H. cameroonense.*

Kaurenoic acid (**2**) [18], 3-acetyloxykaurenoic acid (**3**) [19], β-sitosterol (**4**) [20], and β-sitosterol glucopyranoside (**5**) [21], were identified by comparison with published data.

Cameroonemide A (**1**), kaurenoic acid (**2**), and 3-acetyloxykaurenoic acid (**3**) were tested for herbicidal, antibacterial, and antifungal activities (Table 1). Kaurenoic acid (**2**) and 3-acetyloxykaurenoic acid (**3**) moderately inhibited the alga *Chlorella fusca*, while kaurenoic acid (**2**)

Table 1. Biological activities of the pure compounds[a] in an agar diffusion test.

Compound	Antialgal Chl	Antifungal Mb	Antibacterial Bm
Kaurenoic acid (**2**)	6	0	11
3-Acetyloxykaurenoic acid (**3**)	6	0	0

Note: [a]*C. fusca* (Chl), *M. violaceum* (Mb), and *B. megaterium* (Bm). Fifty micrograms of the substance were applied to a filter disc and sprayed with the respective test organism. The radius of zone of inhibition was measured in mm.

showed strong antibacterial activity against *Bacillus megaterium*. Cameroonemide A (**1**) was inactive in this test.

3. Experimental

3.1 General experimental procedure

Optical rotation was recorded on a Perkin-Elmer 241 MC polarimeter at the sodium D-line. IR spectra were obtained from Nicolet-510P spectrophotometer; ν_{max} in cm^{-1}. EI-MS and HR-EI-MS were carried out using MAT 8200 and Micromass LCT mass spectrometers, in *m/z*. The ^{1}H NMR spectra were recorded on Bruker AMX-500 instruments using TMS as an internal reference. The chemical shifts were reported in ppm (δ), and the coupling constants (J) in Hertz. The ^{13}C NMR spectra were recorded at 125 MHz on the same instrument.

CC was carried out using silica gel (70–230 and 230–400 mesh; E-Merck, Darmstadt, Germany) and Sephadex LH-20 (Amersham Biosciences AB, Uppsala, Sweden). Aluminum sheets precoated with silica gel 60 F 254 (0.2 mm thick; E-Merck) were used for TLC to check the purity of the compounds and were visualized under UV light (254 and 366 nm) followed by ceric sulfate used as the spray reagent.

3.2 Plant material

The plants of *H. cameroonense* were collected at Buea area, southwest (Cameroon mountain), during November 2005, and identified by Mr Elias Ndive (plant taxonomist). A voucher specimen (No. 29191/SRF/CAM) has been deposited at the Herbarium of the Limbé Botanic Garden.

3.3 Extraction and isolation

All parts of *H. cameroonense* plants (6.5 kg) were macerated in MeOH–CH_2Cl_2 at room temperature for 48 h and then filtered. The filtrate was concentrated under vacuum to give 125 g of crude residue. The crude fraction (125 g) was then subjected to CC (silica gel, *n*-hexane, *n*-hexane–EtOAc, and EtOAc, in order of increasing polarity) yielding 11 fractions. Column fraction F_7 (120 mg) [*n*-hexane–EtOAc (2:8)] was similarly subjected to CC, yielding cameroonemide A (**1**, 10.4 mg). Similarly, fraction F_1 (220 mg), eluted with a mixture of *n*-hexane–EtOAc (9.5:0.5), gave kaurenoic acid (**2**, 15.0 mg), while fraction F_2 (350 mg) (*n*-hexane–EtOAc 8:2) gave 3-acetyloxykaurenoic acid (**3**, 8.0 mg) and β-sitosterol (**4**, 10.1 mg). Finally, fraction F_9 (120 mg) gave β-sitosterol glucopyranoside (**5**, 10.2 mg) on subjecting it to CC using MeOH–EtOAc (0.5:9.5) as the eluent.

*3.3.1 Cameroonemide A (**1**)*

Colorless powder, m.p. 137°C. $[\alpha]_D^{20}$ +11.03 ($c = 0.92$, $CHCl_3$ + MeOH). IR ν_{max} (KBr): 3600, 3434, 2930, 2850, 1656, 1510, 1465, 1297 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD): δ (ppm) 0.70 (t, $J = 6.5$ Hz, 6H, H-22′, H-21), 1.20–1.25 (m, H-7–9, H-16–20, H-4′–21′), 1.58 (m, 4H, H-10, H-15), 1.70 (m, 2H, H-3′), 1.73 (m, 2H, H-6), 1.86 (m, 2H, H-5), 1.95–2.10 (m, 4H, H-11, H-14), 3.30 (m, 1H, H-4), 3.35 (m, 1H, H-3), 3.50 (dd, $J = 4.5$, 10.5 Hz, 1H, H-1b), 3.60 (dd, $J = 4.5$, 10.5 Hz, 1H, H-1a), 3.79 (m, 1H, H-2′), 3.91 (m, 1H, H-2), 5.11 (m, 1H, H-13), 5.16 (m, 1H, H-12), 7.35 (d, $J = 8.9$ Hz, 1H, NH). ^{13}C NMR (125 MHz, $CDCl_3$): δ (ppm) 14.7 (C-22′, C-21), 23.4 (C-19), 26.3 (C-21′, C-20), 27.1 (C-20′), 30.1 (C-7–10, C-15–18, C-4′–19′), 32.6 (C-6), 33.4 (C-5), 32.3 (C-11), 32.1 (C-14), 34.7 (C-3′), 53.5 (C-2), 62.5 (C-1), 73.0 (C-2′), 73.5 (C-4), 77.4 (C-3), 131.2 (C-20), 131.3 (C-19), 175.7 (C-1′). EI-MS (EI, 230°C): *m/z* (%) 695.5 (14) $[M]^+$, 354 (33), 339.2 (80), 314 (22), 297.1 (42), 281.1 (58), 237 (20), 139 (33),

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113 (70). HR-EI-MS: *m/z* 695.6442 (calcd for $C_{43}H_{85}NO_5$, 695.6426).

3.3.2 Bioactivity tests: agar diffusion test

The tested compounds (**1**–**3**) were dissolved in acetone at a concentration of 1 mg/ml. Fifty microliters of the solution were pipetted onto a sterile filter disc placed onto an appropriate agar growth medium [22] for the respective test organism and subsequently sprayed with a suspension of the test organism. The test organisms were *B. megaterium* (NB), *Microbotryum violaceum* (MPY), and *C. fusca* (MPY). The radius of the zone of inhibition was measured in mm.

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