*SHORT REPORT*

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# Cameroonenoside A: A New Antialgal Phenolic Glycoside from *Helichrysum cameroonense*

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**Abstract:** *Helichrysum cameroonense* is known for its medicinal value. This paper deals with a phytochemical investigation of this species, from which cameroonenoside A (**1**), a new cinnamic acid glycoside ester has been isolated. Its structure was determined by comprehensive analyses of its $^{1}H$ and $^{13}C$ NMR, COSY, HMQC, and HMBC spectroscopic, and HREIMS mass spectrometric data. Preliminary studies showed that cameroonenoside A (**1**) showed algicidal activity against *Chlorella fusca.*

**Keywords:** Cinnamic acid glycoside ester; *Helichrysum cameroonens;* algicidal.

## 1. Plant Source

In the course of phytochemical studies of medicinal plants from Africa [1-6], we investigate *Helichrysum cameroonense* Hutch. & Dalziel (Asteraceae). We report on the structure elucidation of the new cinnamic acid glycoside ester cameroonenoside A (**1**) (Figure 1).

The plant of *H. cameroonenses* 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 Limbe Botanic Garden.

## 2. Previous Studies

Diterpenes such as kaurenoic acid, 3-acetyloxykaurenoic acid and cermamide have been isolated from *H. cameroonenses* [2].

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## 3. Present Study

All parts of *H. cameroonense* plant was macerated in MeOH-$CH_2Cl_2$ (50:50) 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 column chromatography (silica gel, *n*-hexane, *n*-hexane-EtOAc and EtOAc, in order of increasing polarity) yielding 4 fractions. Fraction $F_3$ was eluted with a mixture of MeOH-EtOAc (1:9) yielding cameroonenoside A (**1**) (10.1 mg).

*Camerooneside A (**1**):* Slighty yellow solid; MP. 179 °C; $[\alpha]_D^{20}$ = – 37.03 (*c* = 0.56, $CHCl_3$ + MeOH); UV ($CHCl_3$): $\lambda_{max}$ (log ε): 305 (3.78), 320 (3.77), 340 (3.65) ; IR $\nu_{max}$ ($CHCl_3$): = 3440, 1690, 1620, 1600, 1020, 1510 $cm^{-1}$; $^1H$ NMR (500 MHz, DMSO-$d_6$): $\delta$ (ppm) = 3.18 (1H, m, H-2'), 3.26 (1H, m, H-3'), 3.34 (1H, m, H-5'), 3.44 (1H, m, H-4'), 3.47 (1H, m, H-6b'), 3.68 (1H, m, H-6a'), 4.91 (1H, d, *J* = 7.5 Hz, H-1'), 5.00 (1H, d, *J* = 5.5 Hz, OH), 5.06 (1H, d, *J* = 4.5 Hz, OH), 5.30 (1H, d, *J* = 5.0 Hz, OH), 5.60 (1H, d, *J* = 2.0 Hz, H-6), 6.24 (1H, d, *J* = 2.0 Hz, H-2), 6.87 (1H, d, *J* = 15.0 Hz, H-*α*'), 7.05 (2H, d, *J* = 8.0 Hz, H-3", H-5"), 7.29 (1H, d, *J* = 15.0 Hz, H-*β*'), 7.59 (d, *J* = 8.0 Hz, 2H, H-2", H-6"); $^{13}C$ NMR (125 MHz, DMSO-$d_6$): $\delta$ (ppm) = 56.8 ($CH_3$, OMe), 61.1 ($CH_2$, C-6'), 70.1 (CH, C-4'), 73.6 (CH, C-2'), 77.0 (CH, C-5'), 77.5 (CH, C-3'), 100.5 (CH, C-2), 101.1 (CH, C-1'), 101.1 (CH, C-6), 116.1 (CH, C-*α*'), 118.2 (CH, C-3", C-5"), 129.3 (C, C-1"), 129.4 (CH, C-2", C-6"), 132.5 (C, C-4), 144.2 (CH, C-*β*'), 158.8 (C, C-5 and C-3), 159.1 (C, C-1), 163.1 (C, C-4"), 171.3 (C, COO); EIMS (rel. int.): *m/z* 464.1 $[M]^+$ (30), 432 (70), 360 (65), 303 (33), 302 $[M\text{-glucose}]^+$ (55), 179 (50), 161 (30), 162 (44), 57 (40), 44 (22); HREIMS: *m/z* 464.1329 (calcd. 464.1318 for $C_{22}H_{24}O_{11}$).

*Bioactivity Test- Agar diffusion test:* The tested compound was dissolved in acetone at a concentration of 1 mg/mL. 50 $\mu$L of the solution were pipetted onto a sterile filter disc, which was placed onto an appropriate agar growth medium [7] for the respective test organism and subsequently sprayed with a suspension of the test organism. The test organisms were *Bacillus megaterium* (NB), *Microbotryum violaceum* (MPY) and *Chlorella fusca* (MPY). The radius of zone of inhibition was measured in mm.

The dried and powdered whole plant of *H. cameroonense* was extracted with MeOH-$CH_2Cl_2$ (50:50). The crude extract obtained after evaporation of the solvent was subjected to conventional purification procedures and resulting in the isolation one new cinnamic acid glycoside ester cameroonenoside A (**1**) (Figure 1).

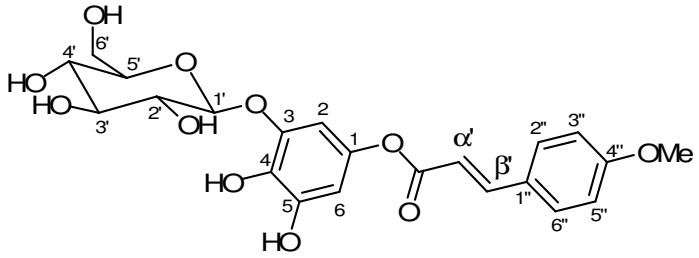
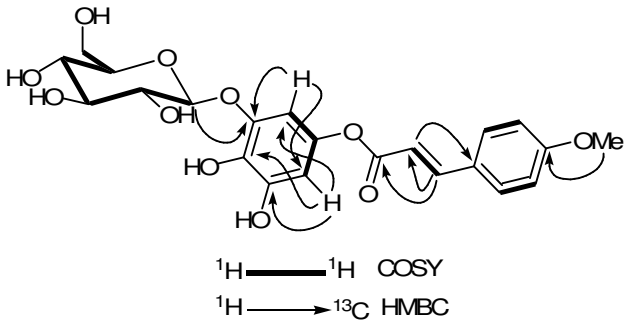



**Figure 1**: Structure of cameroonenoside A (**1**) isolated from *H. cameroonense.*

Compound **1,** cameroonenoside A, was isolated as yellowish needles, for which the UV spectrum showed $\lambda_{max}$ at 340, 320 and 305 nm, indicating its phenolic nature. The EIMS of **1** revealed a molecular weight of 464, consistent with the molecular formula $C_{22}H_{24}O_{11}$, which was confirmed by HREIMS analysis. This was in good agreement with the observation of the one methoxy, one methylene, 13 methine, and seven quaternary carbon resonances in its $^{13}C$ NMR and DEPT spectra. The IR spectrum of **1** showed absorption bands for hydroxyl groups (3440 $cm^{-1}$), *α*,*β*-unsaturated esters ($\nu_{C=O}$ 1690, $\nu_{C=C}$ 1620, $\nu_{C-O}$ 1020 $cm^{-1}$), and an aromatic moiety (1600, 1510 $cm^{-1}$). The $^1H$ NMR spectral data of **1** confirmed one (*E*)-*p*-methoxycinnamic acid moiety in the molecule, as represented by the $A_2B_2$ spin system for four protons of one *para*-substituted aromatic ring [$\delta$ = 7.59 (d, *J* = 8.0

Hz, H-2" and H-6"), 7.05 (d, $J$ = 8.0 Hz, 2H, H-3", H-5")], two *trans* olefinic protons at $\delta$ = 7.29 (d, $J$ = 15.0 Hz, H-$\beta$'), 6.87 (d, $J$ = 15.0 Hz, H-$\alpha$'), and three protons of one methoxy groups at $\delta$ = 3.88 (s, $OCH_3$). This was further supported by the significant fragment at $m/z$ = 161 [methoxy cinnamoyl ion]$^+$. Many other proton signals were observed around 3.1–5.0 ppm, as well as four exchangeable OH protons in $^1$H NMR, indicated the presence of one of a sugar moiety in **1**. This is further confirmed by carbon resonances appearing at $\delta$ = 61.1 ($CH_2$), 70.1 (CH), 73.6 (CH), 77.0 (CH), 77.5 (CH), and 101.1 (CH) in the $^{13}$C NMR, and revealing the presence of a $\beta$-glucopyranoside. The anomeric proton at $\delta$ = 4.91 with coupling constant of $J$ = 7.5 Hz correlated to the carbon signal at $\delta$ = 101.1 in the HMQC spectrum confirming the $\beta$-configuration of the glucoside unit and α-orientation of the proton in the glucose moiety. The $^1$H NMR spectrum also showed signals resulting from an additional tetrasubstituted benzene [$\delta$ = 6.24 (d, $J$ = 2.0 Hz), 5.60 (d, $J$ = 2.0 Hz)]. The position of attachment of the benzene ring was confirmed as the 1-position of the sugar moiety by the NOE correlation between H-1' and H-2 in the NOE spectra of **1**. This was further supported by the HMBC correlation of H-1' to C-3 (Figure 2). Further regiochemistry of compound **1** was confirmed by following HMBC correlation: H-2 with C-1, C-3, C-4, and C-6; H-6 with C-1, C-2, C-4, and C-5; and H-1' with C-3. Thus, the structure of **1** was assigned as cameroonenoside A, as illustrated in Figure 1.




**1**

**Figure 2**. Important $^1$H-$^1$H-COSY and HMBC correlations for cameroonenoside A (**1**).

Cameroonenoside A (**1**) was tested in an agar diffusion assay for their antifungal, antibacterial, and algicidal properties toward *Microbotryum* V*iolaceum, Escherichia coli, Bacillus megaterium*, and *Chlorella fusca* (Table 1). Cameroonenoside A (**1**) moderately inhibited the alga *Chlorella fusca*.

**Table 1**. Biological activity of the pure Cameroonenoside A (**1**) in an agar diffusion test

| Compound | antibacterial | antibacterial | antialgal | antifungal |
|---|---|---|---|---|
| | Chl | Bm | Ec[a] | Mv |
| **1** | 6 | 0 | 0 | 0 |
| Penicillin | 14 | 18 | 0 | 0 |
| Tetracycline | 18 | 18 | 10 | 0 |
| Nystatin | 0 | 0 | 0 | 20 |
| Actidione | 0 | 0 | 35 | 50 |
| Acetone | 0 | 0 | 0 | 0 |

[a]*Chlorella fusca* (Chl), *Microbotryum violaceum* (Mv), *Escherichia coli* (Ec), and *Bacillus megaterium* (Bm). Application of pure substances at a concentration of 0.05 mg (50 µL of 1 mg/mL). The radius of zone of inhibition was measured in mm.

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