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 ¹H and ¹³C 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 cameroonense; 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 ceramide have been isolated from H. cameroonenses [2].

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3. Present Study

All parts of *H. cameroonense* plant was macerated in MeOH-CH₂Cl₂ (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 F₃ was eluted with a mixture of MeOH-EtOAc (1:9) yielding cameroonenoside A (10.1 mg).

*Cameroonenoside A (I):* Slightly yellow solid; MP. 179 °C; [α]D¹⁰ = -37.03 (c = 0.56, CHCl₃ + MeOH); UV (CHCl₃): λ max (log ε): 305 (3.78), 320 (3.77), 340 (3.65) ; IR ν max (CHCl₃): = 3440, 1690, 1620, 1600, 1020, 1510 cm⁻¹; ¹H NMR (500 MHz, DMSO-d₆): δ (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-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 = 15.0 Hz, H-3'), 6.87 (1H, d, J = 7.5 Hz, H-1'), 7.29 (1H, d, J = 15.0 Hz, H-β'), 7.59 (d, J = 8.0 Hz, 2H, H-2', H-6''); ¹³C NMR (125 MHz, DMSO-d₆): δ (ppm) = 56.8 (CH₃, OMe), 61.1 (CH₂-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+glucose]+ (55), 179 (50), 161 (30), 162 (44), 57 (40), 44 (22); HREIMS: m/z: 464.1329 (calcd. 464.1318 for C₂₂H₂₃O₁₁).

**Bioactivity Test- Agar diffusion test:** The tested compound was dissolved in acetone at a concentration of 1 mg/mL. 50 µL 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-CH₂Cl₂ (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 λ 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 C₂₂H₂₃O₁₁, which was confirmed by HREIMS analysis. This was in good agreement with the observation of the one methoxy, one methylen, 13 methine, and seven quaternary carbon resonances in its ¹³C NMR and DEPT spectra. The IR spectrum of 1 showed absorption bands for hydroxyl groups (3440 cm⁻¹), α,β-unsaturated esters (νC=O 1690, νC=C 1620, νC-O 1020 cm⁻¹), and an aromatic moiety (1600, 1510 cm⁻¹). The ³H NMR spectral data of 1 confirmed one (E)-p-methoxycinnamic acid moiety in the molecule, as represented by the A₂B₂ spin system for four protons of one para-substituted aromatic ring [δ = 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 δ = 7.29 (d, J = 15.0 Hz, H-β), 6.87 (d, J = 15.0 Hz, H-α), and three protons of one methoxy groups at δ = 3.88 (s, OCH₃). 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 ¹H NMR, indicated the presence of one of a sugar moiety in 1. This is further confirmed by carbon resonances appearing at δ = 61.1 (CH₂), 70.1 (CH), 73.6 (CH), 77.0 (CH), 77.5 (CH), and 101.1 (CH) in the ¹³C NMR, and revealing the presence of a β-glucopyranoside. The anomeric proton at δ = 4.91 with coupling constant of J = 7.5 Hz correlated to the carbon signal at δ = 101.1 in the HMQC spectrum confirming the β-configuration of the glucoside unit and α-orientation of the proton in the glucose moiety. The ¹H NMR spectrum also showed signals resulting from an additional tetrasubstituted benzene [δ = 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 camerooneside A, as illustrated in Figure 1.

![Figure 2. Important ¹H-¹H-COSY and HMBC correlations for cameroonenoside A (1).](image-url)

Cameroonenoside A (1) was tested in an agar diffusion assay for their antifungal, antibacterial, and algicidal properties toward Microbotryum violaceum, 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**

<table>
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<tr>
<th>Compound</th>
<th>antibacterial</th>
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<th>antifungal</th>
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<tr>
<td>Acetone</td>
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</table>

³Chlorella 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.
References


